POSTER SESSION II

Myeloma and other monoclonal gammopathies IV

0751

THE RISK OF THROMBOCYTOPENIA AND NEUROPATHY AFTER BORTEZOMIB THERAPY DEPENDS ON THE BASELINE PLATELET COUNTS AND PREVIOUS NEUROPATHY RESULTS OF CZECH MYELOMA GROUP

I.S. Spicka

General Fac. Hosp. Prague, Prague, Czech Republic

Thrombocytopenia and neuropathy are the most frequent and serious complications of bortezomib therapy. However, previous data suggested that the risk of these adverse events depends on the baseline involvement associated mostly with previous therapy. Bortezomib as monotherapy was given to 82 consecutive patients with refractory/relapsed multiple myeloma in 5 centers in Czech republic. Thrombocytopenia and neuropathy were, of course, the most frequent complications, observed in 65,9% and 52,4% of patients, respectively. In 63 patients baseline platelet counts were within normal range, in 38 of them thrombocytopenia developed during the course of therapy. Nineteen patients had mild thrombocytopenia before the start of bortezomib treatment. The risk of thrombocytopenia was 60,3% (grade 3/4 thrombocytopenia 30,2%) in patients with normal baseline values and 84,2% (gr. 3/4 63,2%) in patients with previous abnormality. Similar relationship was observed in the case of neuropathy. The risk of neuropathy was 45,2% in patients without any signs of neurological involvement before the start of therapy compared with 75% risk in the patients with grade 1 of any type of neuropathy in the history. Grade 3/4 neuropathy occurred in 1,6% of patients in the first group and in 25% in the second one. *Conclusions.* Thrombocytopenia and, especially, neuropathy could lead to dose adjustment and/or premature termination of bortezomib therapy in myeloma patients. The association between the risk of these complications and the baseline involvement supports the statement that it would be advantageous to start treatment with bortezomib earlier in the course of disease.

0752

BORTEZOMIB (VELCADE) IN COMBINATION WITH LIPOSOMAL DOXORUBICIN (DOXIL) AND THALIDOMIDE IS AN ACTIVE SALVAGE REGIMEN IN PATIENTS WITH RELAPSE OR REFACTORY MULTIPLE MYELOMA: FINAL RESULTS OF A PHASE II STUDY

S. Padmanabhan, K. Miller, L. Musiel, Z. Bernstein, M. Czuczman, J. Yu, A. Chanan-Khan

Roswell Park Cancer Institute, Buffalo, NY, USA

Background. Tumor microenvironment (ME) plays an important role in MM. It is associated with disease progression, metastasis, and resistance to therapy. Therefore, targeting the ME and the tumor cell simultaneously may be an effective way to overcome resistance in pts with rel/ref MM. *Aims*. Orlowski *et al*. reported improved anti-tumor responses when bortezomib (V) was combined with doxil (D) in pts with hematologic malignancies. We investigated clinically, this approach i.e., targeting the MM cell as well as its ME, using a combination of V, D and low-dose thalidomide (T) as salvage therapy for pts with rel/ref MM. Here we report the final results of this phase II study. *Methods.* All pts with rel/ref disease were eligible for this study. V was given at 1.3mg/m^2 (D1,4,15,18) and D at 20mg/m^2 (D1,15) every 4 weeks with daily T (200 mg) for 4-6 cycles. SWOG criterion was used to assess response. Low-dose coumadin (1-2 mg po qd) was used for prevention of venous thromboembolism (VTE). *Results.* Twenty three pts (9M, 14F; median age -58 , range 43-80 yrs; 21MM, 2 WM) have been enrolled to date. All pts had Stage III disease, median b2M was 3.8 and median number of prior therapies were 3 (range 1-7). Prior therapies included stem cell transplant(SCT) in (41%), T (41%), adriamycin(A) (65%) steroids (82%) and velcade (12%). 74% had refractory disease. Seventeen pts have completed at least 1 cyc and are available for toxicity and response evaluation. One pt died of sepsis prior to completing 1 cyc and 1 pt with PR was taken off study for non-compliance after 1 cyc. ORR was with 65%(CR+PR) with 23% CR all of whom were IFE negative. Toxicity: 2 pts developed Gr. II plantar-palmar erythrodysthesia (PPE) and 1 had Gr. III cellulitis. No VTE was noted. No significant non-hematologic Gr. III/IV toxicity were seen. Despite prior exposure to anthracycle, we did not noted any cardiotoxicity with D. *Conclusions.* Pt with rel/ref MM usually have aggressive disease with paucity of effective regimens. VDT is a highly active salvage regimen that demosntrates high response rates including CR and acceptable toxicity in patients with relapsed/refractory multiple myeloma.Responses were noted despite prior failure of steroids, T ,A and even V. VTE does not appear to be a problem with this low dose coumadin prophylaxis. Final results of this phase II study will be presented at the annual EHA meeting.

0753

FLOW CYTOMETRIC IMMUNOPHENOTYPIC ANALYSIS OF 25 CASES OF PLASMA CELL LEUKEMIA

M. Kraj, R. Poglod, J. Kopec-Szlezak, K. Owczarska

Institute of Haematology and Blood Trans, WARSAW, Poland

The aim of the study was to determine expression of adhesion molecules CD11a (LFA-γ), CD18 (LFA-1γ), CD11b, CD29, CD49d, CD44 (H-CAM), CD54 (ICAM-1), CD56 (N-CAM) and CD117 (c-kit) on peripheral blood (PB) and bone marrow (BM) lymphoid cells in 25 plasma cell leukemia patients (PCL), at diagnosis and in the control group of 10 healthy subjects. Immunophenotyping was performed on freshly collected blood and bone marrow samples by means of flow cytometry. Plasma cells were identified as showing high-density expression of CD38 and CD138 (syndecan-1). *Results*. of analysis were presented both as relative and absolute (omitted in abstract) values of numbers of cells with antigen expression and as relative fluorescence indices (RFIs) of studied antigens. Statistical analysis was performed using Wilcoxon's test. All below presented differences are statistically significant. The study revealed in PCL patients a significantly higher relative and absolute number of CD54+ cells (in brackets: means±SD of PCL pts vs control) both in BM (63±29% vs 13±5%) and PB (49±25% vs 8±3%) as well as that of CD38+ cells both in BM (84±12% vs 54±11%) and PB (74±11% vs 52±7%). In turn, PCL patients showed a decreased relative number of BM: CD11a+cells (40±28% vs 73±10%), CD18+cells (47±25% vs 88±7%), CD11a+CD18+cells (42±27% vs 72±10%), CD44+cells (71±26% vs 93±4%), CD11b+cells (17±12% vs 35±10%) and PB: CD11a+cells (58±28% vs 96±3%), CD18+cells (58±29% vs 99±0,2%), CD11a+CD18+cells (58±29% vs 96±3%), CD44+cells (86±15% vs 98±0,9%). In BM of PCL patients compared with the control there were found decreased RFIs of CD18 (15,0±1,3 vs 16,6±0,7) and CD29 (8,6±1,4 vs 10,4±0,8) and increased RFIs of CD54 (16,9±2,5 vs 13,0±0,5) and CD11a (18,4±1,5 vs 14,7±0,9). In PB of PCL patients RFIs of CD29 (10,4±1,2) was lower than this in control (11,6±0,9) while RFIs of CD38 (16,9±3,0 vs 14,8±1,3), CD54 (16,1±2,8 vs 12,3 ±0,3), CD11a (20,4±1,8 vs 18,3±0,8) were higher. BM leukemic cells with strong CD38 expression and CD138 expression showed antigen coexpression in following number of cases: CD54 in 16/19 (82% tested), CD29 in 12/12 (100%), CD49d in 9/9 (100%), CD44 in 9/11 (82%), CD11a in 3/20 (15%), CD11b in 3/12 (25%), CD18 in 2/17 (11%), CD56 in 13/23 (56%), CD117 in 5/13 (38%) and CD19 in 0/13 (0%) tested cases. PB leukemic cells showed coexpression of CD54 in 17/19 (89%), CD29 in 12/13 (92%), CD49d in 11/11 (100%) CD44 in 9/10 (90%), CD11a in 9/20 (45%), CD11b in 8/12 (66%), CD18 in 11/17 (64%), CD56 in 13/22 (59%), CD117 in 5/13 (36%), CD19 in 0/16 (0%) of tested cases. *Conclusions*. Immunophenotype of leukemic plasma cells characterizes mainly increased expression of CD38, CD54 and CD138 also expression of CD29, CD49d, CD44 and disturbed expression of CD18, CD11a and CD11b. In one half cases tumor cells show expression of CD56 and CD117.

0754

INORGANIC POLYPHOSPHATE IS PRESENT AND INDUCES APOPTOSIS SPECIFICALLY IN HUMAN PLASMA CELLS

A. Ruiz,[1] L. Hernandez-Ruiz,[1] I. Gonzlez-Garca,[1] C. Castro,[2] J.A. Brieva[1]

[1]*Hospital U. Puerta del Mar, Cadiz, Spain;* [2]*Universidad de Cadiz, Cadiz, Spain*

Backgrounds. Inorganic polyphosphate (polyP), a ubiquitous phosphate polymer with ATP-like bonds, participates in a variety of functions including blood coagulation and cell proliferation. Recently, we have reported that human platelets have massive quantities of polyP accumulated in dense acidic granules known as acidocalcisomes, in a similar way to that occurring in unicellular microorganisms (*Ruiz et al. J Biol Chem 2004: 279:44250*). *Aims*. We have investigated here the presence of polyP in human plasma cells (PC), responsible for the production and maintenance of antibodies in response to antigens. We also study the effects of extracellular polyP in the biology of the human PC. *Methods*.We measured levels of polyP in the U266 and IM9 myeloma cell lines by a

specific enzymatic assay. The localization of polyP in the myeloma cell lines was determined by confocal microscopy. The U266 myeloma cell line was used to study whether extracellular polyP affects Ig secretion and survival. Different human cell lines were used to test the specificity of polyP in viability. We analyzed Ig secretion of PC form Bone Marrow and Peripheral Blood after polyP addition. A conventional tetanus toxoid booster immunization was used to increase PC proportion in order to examine the apoptotic effects of polyP. Ig secretion and Apoptosis was determined by ELISA and FACS respectively. *Results*. Micromolar levels of polyP that is present principally as polymers of 75 phosphate units have been found in the U266 and IM9 myeloma cell lines. PolyP is accumulated in intracellular vacuoles similar to the previously reported platelet dense granules and acidocalcisomes of the unicellular eukaryotes. Addition of polyP to human PC produces an unexpected inhibition of Ig secretion and a stimulation of apoptosis. PolyP generates apoptosis specifically in PC, myeloma (malignant PC) cell lines, and B lymphoid cell lines. Normal B cells, T cells, total blood mononuclear cells, and non-lymphoid cell lines are not affected by polyP. In U266 myeloma cell line, polyP induces the externalization of phosphatidylserine, the activation of caspase-3, and the arrest of the cell cycle. Protective effects of IL-6 do not overcome the polyP-induced apoptosis. *Summary/conclusions*. Taken together, our results and suggest for the first time the relevance of polyP for the humoral immune response and open prospects for polyP as a novel therapy for myeloma.

0755

METHYLATION STATUS OF THE P57KIP2 GENE IN PATIENTS WITH PLASMA CELL DYSCRASIAS

E. Hatzimichael,[1] A. Dasoula,[2] A. Makis,[1] M. Syrrou,[2] K. Bourantas[1]

[1]*Haematology Clinic, Ioannina, Greece;* [2]*Laboratory of General Biology, Ioannina, Greece*

Background. Oncogenesis is related to cell cycle deregulation. Aberrant DNA methylation, leading to silencing of regulatory genes, has emerged as one of the most frequent molecular changes in haematological malignancies. The p57KIP2 is a tumor suppressor gene that belongs to the CIP/KIP family of cyclin dependent kinase inhibitors that negatively regulate cell cycle progression. *Aim*. We have studied the methylation status of the promoter region of p57KIP2 gene in patients with multiple myeloma (MM) and Waldenström's macroglobulinemia (WM) in order to correlate the methylation pattern with the disease's phenotype. *Patients and Methods.* We have studied bone marrow and paired peripheral blood samples from 12 consecutive MM patients (9 male, 3 female, age range 50-83, median 59) and 2 consecutive WM patients (1 male and 1 female, age 75 and 47 years).

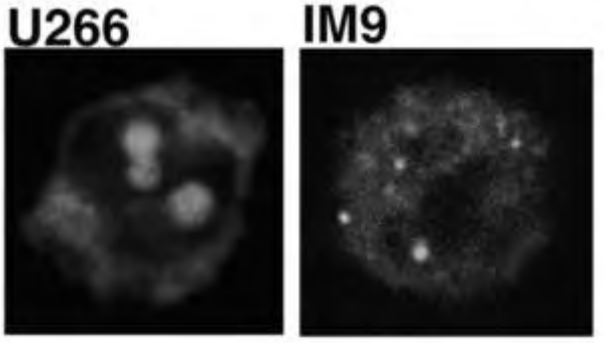


Figure 1. Localization of polyP on U266 and IM9 cells.

Samples from 9/12 MM patients and 2/2 WM patients were taken at diagnosis whereas the remaining 3/12 samples were taken during the course of the disease. Genomic DNA was extracted using standard protocols (Quiamp DNA mini kit). After bisulfite treatment procedure the DNA was PCR amplified with primers specific for the methylated and the unmethylated alleles of the gene. The PCR products were separated on 2% agarose gel. Bone marrow DNA from healthy donors served as negative control. We have also used human male genomic DNA universally methylated for all genes (Intergen Company, Purchase, NY) as positive control. *Results*. Two patients had stage IA disease and did not receive any treatment, five MM patients had stage IIA or more advanced disease and started on VAD chemotherapy, two patients were started on oral melphalan and methylprednisolone, one patient was on plateau, and two patients had progressive disease after having received VAD and were started on bortezomib therapy. One patient with WM was started on cyclophosphamide, dexamethasone and rituximab and the other patient did not receive any treatment. Classical cytogentic analysis was available on 5/12 MM and 1/2 WM patients and the karyotype was reported as normal. All patient samples showed no band corresponding to the methylated allele of the p57KIP2 gene. The band corresponding to the unmethylated allele was clearly visible in all samples. *Conclusion*. To our knowledge this is the first report on p57KIP2 methylation status in patients with plasma cell dyscrasias. Our data show that methylation of p57KIP2 gene is not a frequent event in the patients studied. Further studies are needed to confirm the above results.

0756

EVALUATION OF THE RELATION BETWEEN ANGIOGENIC CYTOKINES, SELECTED BIOLOGICAL PARAMETERS AND PROGNOSTIC FACTORS IN MULTIPLE MYELOMA

V.S. Scudla,[1] T.P. Pika,[1] M.B. Budikova,[2] M.O. Ordeltova,[3] J.M. Minarik,[1] M.Z. Zemanova,[1] J.B. Bacovsky,[1] K.S. Srovnalik[4]

[1,3rd] *Department of Internal Medicine, Olomouc, Czech Republic;* [2]*Department of Nuclear Medicine, Czech Republic;* [3]*Department of Clinical Immunology, Olomouc, Czech Republic;* [4]*Department of Haematology, Vsetin, Czech Republic*

Background. Multiple myeloma is an unusually heterogenous disease with individually different course, response to therapy and prognosis. Up-to-date diagnostic and stratification systems have, however, an important limitation in their insufficient absorption of those parameters, that express intrinsic biological properties of myeloma cells and the microenvironment of the bone marrow. The aim of this study was to evaluate the relation of 10 biological parameters to 6 substantial prognostic factors in multiple myeloma. *Methods*. The analysed group consisted of 66 persons evaluated at the time of diagnosis, before the start of chemotherapy. For the assessment of serum levels of examinated molecules were used following Methods. REA, RIA, ELISA and the technique of sandwich enzymatic immunoassay, for the assessment of proliferative and apoptotic properties were used propidium iodide (PC-PI) and annexin V (PC-AI) indices evaluated with the help of flow-cytometry. Statistical analysis was carried out using Pearson and Spearman test and/or using U-test according to Mann-Whitney. *Results*. High occurence of abnormal serum level of evaluated parameter was found in the case of S-β-2-microglobulin (95,5%), S-thymidinekinase (57,5%), S-sVCAM-1 (78,5%), S-ICTP (87,0%), S-soluble osteoprotegerin (sOPG 76,5%) , S-sSyndecan-1 (56,5%) and low index of apoptosis of plasma cells (PC-AI, 78%). Correlation analysis (Pearson test) revealed a mutual relationship between serum levels of β-2-microglobulin to sVCAM-1 (r=0,39, *p*=0,002), sICAM-1 (r=0,33, *p*=0,011), sOPG (r=0,53, *p*=0,001), sHGF (r=0,34, p=0,006), sSyndecan-1 (r=0,38, *p*=0,003) and sFas (r=0,42, *p*=0,001); of S-albumin to sVCAM-1 (r=-0,29, *p*=0,036), ICTP (r=-0,33, *p*=0,016), sOPG (r=-0,63, p=0,000), sHGF (r=-0,39, p=0,003) and sSyndecan-1 (r=-0,29, *p*=0,042; of S-thymidinekinase to sSyndecan-1 (r=0,46, *p*=0,000) and sFas (r=0,29, *p*=0,019). In neither of the cases was found the relation of PINP and VEGF to any of the evaluated prognostic factors. There was no relation found between any of the analysed parameters and PC-PI or PC-AI. With the use of U-test there was found a relationship of serum levels of sIL-6R (< > 100IU/l) to β-2-microglobulin (*p*=0,001), albumin (p=0,002) and to PC-PI (*p*=0,046). *Conclusion*: The above study established the possibility to enrich the traditional algorithms used in clinical practice for individual characteristics of MM with the parameters sOPG, sHGF, sSyndecan-1 and sFas.

Founded by MSM 6198959205.

0757

COMPARISON OF SERUM LEVELS OF BIOLOGICAL PARAMETERS IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND MULTIPLE MYELOMA

V.S. Scudla,[1] T.P. Pika,[1] M.B. Budikova,[2] J. M. Minarik,[1] M.Z. Zemanova,[1] J.B. Bacovsky,[1] V.H. Heinzova[#]

[1,3rd] *Department of Internal Medicine, Olomouc, Czech Republic:* [2]*Department of Nuclear Medicine, Olomouc, Czech Republic;* [3]*Department of Haematology, Opava, Czech Republic*

Background. The presented work is focused upon the evaluation of the differences between serum levels of selected biological parameters in monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM), especially from the point of view of their potential benefit for clinical practice. *Methods*. Analysed group of 96 patients (30 patients with MGUS and 66 patients with MM) was assessed at the time of diagnosis before the start of therapy. For the

evaluation of serum levels of analysed parameters were used following Methods. radioenzymatic assay (thymidinekinase), radioimmunoanalysis (β-2-microglobulin, ICTP, PINP), method of enzymoimmunoassay (sIL-6R, sVCAM-1, sICAM-1, sOPG and sRANKL) and the technique of quantitative sandwich enzymatic immunoassay (sHGF, sVEGF, bFGF, syndecan-1/CD138 and sFas). Statistical analysis was carried out using Pearson´s and Fischer´s test, χ^2 test and nonparametric U test according to Mann-Whitney (p<0,05). Results. Statistically significant differences were found out between MGUS and MM in case of comparison of serum levels of sIL-6R (p=0,02), ICTP (p=0,001), sHGF (p=0,001) and syndecan-1/CD138 (p=0,001), whereas in case of sVCAM-1, sICAM-1, PINP, sOPG, sVEGF and sFAS there were no statistically significant differences. Within the analysis of the frequency of the occurence of abnormal values in the MM and MGUS group there were significant differences not only in the case of standard parameters such as β-2-microglobulin, thymidinekinase creatinine and albumin, but also in the case of sIL-6R, ICTP, sHGF, and syndecan-1, however not in the case of comparison of the values of sVCAM-1, sICAM-1, PINP, sOPG, sVEGF, and sFas. Measurement of serum levels of sRANKL and soluble form of bFGF was of no avail due to very low values of these parameters. Conclusion: The analysis of the 10 parameters, that are altogether very close related to the biological properties of clonal plasma cells or to the changes of bone marrow microenvironment revealed from the point of the contribution for the differentiation of MGUS from MM, that the only purposeful parameters were only the serum levels of sIL-6R, ICTP, sHGF and syndecan-1 (sCD138), i.e. the parameters with certified significance for the MM prognosis evaluation.

Founded by MSM 6198959205.

0758

RELATIONSHIP OF SERUM FREE LIGHT CHAIN LEVELS TO DEGREE OF MULTIPLE MYELOMA PROGESSION

T.P. Pika, V.S. Scudla, J.M. Minarik

3rd Department of Internal Medicine, Olomouc, Czech Republic

Backround: Multiple myeloma is a malignant disease characterised by clonal proliferation and accumulation of neoplastically transformed B-line elements, producing monoclonal immunoglobulin (MIG) demostrable in serum and/or urine. Plasma cells also produce free light chains (FLC) κ and λ, that are not fixed in MIG molecule. Aim of the study was a comparison of serum FLC levels and κ/λ (κ/λ) between stages of Durie' Salmon (D-S), International Prognostic Index (IPI) and South West Oncology Group (SWOG) staging systems. *Methods.* Prospective study included 147 patients with multiple myeloma, examined during one year period. Serum FLC levels were assesed using FREELITE Immunotech system, values of β2-microglobulin were obtained by RIA. Mann - Whitney's U-test was used for statistic evaluation. *Results.* Abnormal values of serum FLC and κ/λ ratio were assesed in 79% and 81%, κ secretion was presented in 67%, λ in 33%. Comparing with each stage of D-S staging system, statistically different levels of dominant chain (p=0,003) and κ/λ ratio (p=0,005) were found between stages II and III in λ group only. Differences in values between other stages were not significant. Comparing substage A and B (serum creatinine over 177 μmol/L), significant differences were found in levels of dominant and alternative chain in κ group (p=0,047 and p=0,014) and also in λ group (p=0,007 and p=0,046), but there was no significant difference between κ/λ ratio values. Using IPI staging system, significantly different levels of dominant kappa chain were found in κ group between stages I and II (p=0,029), between stages I and III in values of κ chain (p=0,029) and κ/λ ratio (p=0,04). In lambda group were also found differences in λ dominant chain and κ/λ ratios between stages I and II (p=0,01 and p=0,011) and also between stages I and III (p=0,0001 and p=0,0013). Differences of FLC values between stages II and III in both groups were not significant. In case of SWOG staging system, in κ group, differences in levels of dominant chain between stages I and II (p=0,038) and I and III+IV (p=0,035) were assesed. In λ group were found different values of dominant chain lambda and κ/λ ratio between stages I and II (p=0,029 and p=0,042) and also between I and III+IV (p=0,002 and p=0,004). Between stages II and III+IV was found a difference in dominant chain in lambda group only (p=0,047). *Conclusions.* Disease progession degree evaluated using dynamic indicators - serum albumine and β2-microglobuline (IPI and SWOG system), correlate with serum FLC levels more expresively, than traditional Durie-Salmon staging system. Serum FLC levels depend on kidney function, but κ/λ ratio values are not affected by impaired renal function.

0759

VEGF EXPRESSION AND MICROVESSEL DENSITY IN PATIENTS WITH MULTIPLE MYELOMA: CLINICAL AND PROGNOSTIC SIGNIFICANCE

O. Markovic,[1] D. Marisavljevic,[2] V. Cemerikic,[3] M. Perunicic,[3] M. Bakrac,[3] A. Vidovic,[3] I. Elezovic,[3] M. Colovic[3]

[1]*Medical Training Center 'Bezanijska Kosa, Belgrade, Serbia and Montenegro;* [2]*Medical Training Center 'Bezanijska Kosa, Belgrade, Serbia and Montenegro;* [3]*Institute of Hematology, KCS, Belgrade, Serbia and Montenegro*

Background. Angiogenesis or new vessel formation is an essential component in the growth and progression of solid malignancy. However, conflicting data are reported on clinical significance of VEGF deregulation and microvessel density (MVD) in multiple myeloma (MM). *Aim*: The purpose of the study was to evaluate the incidence of VEGF expression and grade of MVD, and to correlate these findings with pathohistological and clinical features of newly diagnosed myeloma patients. *Patients and methods*. We analyzed bone marrow biopsy specimens obtained from 59 patients with MM. The clinical staging was done according to the Durie and Salmon classification (four patients had disease stage I, 15 patients stage II and 39 patients stage III). Expression of VEGF and MVD were analyzed using standard imunohistochemical analysis of B5-fixed and routinely processed, paraffin-embedded bone marrow specimens with antibodies against VEGF and CD34, respectively. MVD was estimated by counting number of microvessels in three *hot spots* at magnification x400, according to the method of Weidner *et al*. VEGF imunoreactivity was estimated on the basis of intensity and percentage of positive plasma cells. *Results*. VEGF was expressed in 47 out of 59 (79.66%) specimens. No statistical correlation could be found between VEGF overexpression and age, clinical stage, degree of osteolytic lesions, types of monoclonal protein, hemoglobin concentration, platelet count, serum concentration of creatinin, calcium and albumins, the extent of bone marrow infiltration, histological grade and proliferative activity (measured with Ki-67 immunoreactivity). In addition, no significant difference regarding overall survival was found between VEGF positive and VEGF negative cases (29 months vs. 34 months, v=0.8). Median MVD was 15 (range: 1-89). We found significant correlation between MVD and histological grade, the extent of bone marrow infiltration and proliferative activity. Although MVD showed prognostic impact on overall survival in univariante analysis (p=0.009), multivariate analysis identified only age, hemoglobin concentration and proliferative activity as independent prognostic factors. *Conclusions*. The upregulated VEGF is seen in plasma cells in the majority of myeloma cases. However, the relationship between this finding and pathogenesis of the disease still remains to be established. The microvessel density can predict poor survival in myeloma and be helpful in choosing optimal therapeutic modality in every individual patient.

0760

CLONOGENIC CAPACITY OF BONE MARROW CELLS IN PATIENTS WITH MULTIPLE MYELOMA. THE INFLUENCE OF ARSENIC TRIOXIDE AND BORTEZOMIB ON THE PROLIFERATION OF CFU-F AND CFU-GM

W.K. Knopinska-Posluszny,1 M.T. Taszner,2 A.H. Hellmann2

[1]*Medical University School Laboratory Diag, Gdansk, Poland;* [2]*Dept. of Haematology, Gdansk, Poland*

Arsenic trioxide (As_2O_3) and bortezomib were tested as therapeutic agents for a variety of malignancies. The aim of our study was to investigate *in vitro* effects of As_2O_3 and bortezomib on clonogenic capacity of haematopoietic and mesenchymal progenitor cells in patients, with newly diagnosed multiple myeloma and patients with multiple myeloma resistant to standard chemotherapy. *Materials and methods*. Bone marrow samples were obtained from 24 patients with multiple myeloma: 10 before treatment and 14 patients resistant to standard chemotherapy, 11 females and 13 males, 16 with IgG, 6 with IgA, 1 with IgD, 1 with BJ myeloma. Mononuclear cells (MNC) were cultured without As2O3 or bortezomib and with As_2O_3 at a concentration of 0,2 mmol/l. and bortezomib at a concentration of 10 and 20 ng/mL. MNC were plated in a standardized methylcellulose medium (MethoCult 4434, StemCell Technologies) and MesenCult (StemCell Technologies). Colony formation of haematopoietic progenitors (CFU-GM and BFU-E) and mesenchymal progenitor cells (CFU-F) based on morphology of the colonies were assessed on day 14 of cultures. CFU-GM, BFU-E and CFU-F expressed as the percentage of decrease versus control and the mean and standard deviation (SD) of colony inhibition for each concentration of As_2O_3 or bortezomib were calculated across all samples. *Results*. In all patients with resistant myeloma and 2/3 of newly diagnosed patients we

observed an increased number of mesenchymal progenitors in cultures. As_2O_3 and bortezomib caused reduction of CFU-GM and BFU-E formation after 14 days of incubation to 1% and 0,5% of control values respectively. Formation of CFU-F was completely inhibited by As_2O_3 and bortezomib. *Conclusions.* Our data clearly demonstrate that in in vitro conditions exposure to As_2O_3 or bortazomib even in low concentration is able to induce growth inhibition of haematopoietic progenitor cells in patients with multiple myeloma. Especially As_2O_3 and bortezomib inhibits completely formation of mesenchymal progenitor cells in this group of patients. A combination of direct toxicity against leukemic cells with proapoptotic activity of bortezomib or As_2O_3 may be the optimal characteristic of a successful antimyeloma agent in particular in patients with increased number of CFU-F before treatment.

0761

CHARACTERIZATION OF THE PLASMA CELLS OF MULTIPLE MYELOMA BY SERIAL ANALYSIS OF GENE EXPRESSION

M. Ortega, A. Cunha, D. Albuquerque, A. Duarte, C. De Souza, I. Lorand-Metze, F. Costa, C. Lima

State University of Campinas, Campinas, Brazil

Backgrounds. In the last years, several large-scale gene expression studies with array based hybridisation have been performed in multiple myeloma (MM) and many genes compromised with the disease have been identified. However, the molecular mechanisms involved in the disease are still not completely elucidated. More recently, the serial analysis of gene expression (SAGE) method has allowed the global analysis of genes expressed in a determined cell or tissue. However, to the best of our knowledge, no consistent studies in plasma cells of MM have already been performed using the SAGE method. *Aim*. The aim of this study was to characterize the plasma cells of MM by SAGE. Methods. Purified normal plasma cells (PNPC) differentiated from bone marrow B cells of a healthy individual and purified neoplasic plasma cells from a newly diagnosed MM patient were obtained by magnetic sorting in a column, using the CD-138 antibody Macs microbeads (MACS, Miltenyi Biotec, Germany). Both SAGE libraries, PNPC and MM, were obtained using the I-SAGE Kit (Invitrogen, Life Technologies, USA), in accord with the manufacturer procedures. The expression of a group of genes arbitrarily selected were further investigated by quantitative polymerase chain reaction real-time (qRT-PCR) in the sample of SAGE MM and in other samples of MM patients, in comparison with SAGE PNPC sample, with the purpose of verify the reliability of the results obtained by SAGE. The functional classification of genes was performed according to the Gene Ontology Consortium. Results. We generated, after automatic sequencing, a total of 84.965 tags from the MM plasma cells and 77.080 tags from the normal plasma cells, representing 24.601 and 25.527 unique tags, respectively. In the comparison of both profiles, 476 differentially expressed transcripts were identified (p<0.01; fold 5). Approximately 70% of the unique tags from both profiles were known genes or annotated sequences, and 30% corresponded to tags that may represent novel transcripts. The expression of 8 up-regulated genes (*CCND1, DUSP1, FOS-B, IGHG3, IGKC, V-FOS, V-JUN, PRDM2*), 6 down-regulated genes (*CD19, CD40, EEF1D, FCER2, IL6-ST e RNAse1*) and two normally expressed genes (B2M e XBP-1) on MM library were evaluated by qRT-PCR in the SAGE MM and in other samples of MM patients. Similar mean expression values were found in both materials. A distinct mean expression value of the *PRDM2* gene (109.51 in MM library vs 1.00 in MM samples) was the unique discordant result. The functional classification of genes revealed abnormal expression of genes involved in transcription, signalling, cell proliferation and apoptosis. Conclusions. We identified abnormal expression of genes involved in fundamental steps of plasma cells proliferation and survival, which may contribute to the comprehension of MM pathophysiology, and to the identification of new targets for MM therapy.

Supported by FAPESP

0762

THE PREVALENCE OF K-RAS AND N-RAS MUTATIONS IN BRAZILIAN PATIENTS WITH MULTIPLE MYELOMA

M. Ortega,[1] R. Faria,[2] A. Assis,[1] D. Albuquerque,[1] J. Oliveira,[2] M. Noguti,[2] J. Faria,[2] F. Costa,[1] C. Lima[1]

[1]*State University of Campinas, Campinas, Brazil;* [2]*Federal University of Sao Paulo, Sao Paulo, Brazil*

Background. Point mutations affecting codons 12, 13 (exon 1) and 61 (exon 2) of the N- RAS gene and codons 12, 13 (exon 1) of the K-RAS gene are identified in about 30.0% and 10.0% of MM patients of the Northern hemisphere, respectively. *Aim.* Since there are no reports about the prevalence of RAS genes mutations in MM Brazilian patients, this was the aim of the present study. Methods. DNA from bone marrow aspirates of 252 patients with MM were investigated for whole exons 1 and 2 of the N-RAS gene and whole exon 1 of the K-RAS gene by direct sequencing of DNA amplified in vitro by the polymerase chain reaction. Results. Fifty-three out of 252 (21.03%) MM patients presented RAS mutations. Heterozygous mutations of the N-RAS gene were found in seven out of 252 (2.78%) patients. Three of them (1.19%) presented a mutation in exon 1, at codon 4 (TAC_AAC), one patient (0.40%) presented a mutation in exon 1, at codon 10 (GGA_GAA), and 2 patients (0.79%) presented a mutation in exon 2, at codon 61 (CAA_CAT). A mutation in exon 2, at codon 65 (AGT_ACT) was identified in one patient (0.40%). Heterozygous mutations of the K-RAS gene at codons 7, 12 and 13 were found in 46 out of 252 (18.25%) patients. Twenty-six of them (10.31%) presented a new mutation at codon 7 (GTG_GCG). One patient (0.40%) presented a mutation at codon 12 (GGT_GTT). A mutation at codon 13 (GGC_AGC) was identified in 19 patients (7.54%). Similar frequencies of the K-RAS gene mutation at codon 7 were observed in patients stratified by age (46.15% in patients ≤60 years vs 53.85% in patients >60 years; p=0.83), gender (57.70% in males vs 42.30% in females; p=0.80), ethnic origin (9.61% in blacks vs 17.69% in caucasians; p=0.24), status (73.08% in patients at diagnosis vs 26.92% in patients at progression of the disease; p= 1.00) and stage of the disease (30.77% in patients at stages I+II vs 69.23% in patients at stage III; p=0.82). There was no difference between the median percentages of the plasma cells obtained from the bone marrow of patients with and without K-RAS gene mutation at codon 7 (26.26% vs 26.20%, p=0.70). Similar median values of serum creatinine (1.90 mg/dl vs 1.19 mg/dL; p=0.16) and lactate dehydrogenase (239.00UI/l vs 207.00UI/l; p=0.21) were also seen in both groups of patients. Fifteen patients who presented mutations at codon 7 of K-RAS gene presented mutations at codon 13, as well (p<0.001). Conclusions. Brazilian MM patients apparently are characterized by: 1) Low prevalence of RAS mutation; 2) RAS mutations located at distinct regions of the critical codons of the N- and K-RAS genes. In addition, since the mutation of the K-RAS gene at codon 13 had low influence on oncogenic transformation of bladder carcinoma patients, the concomitancy of mutations at codons 7 and 13 in 15 MM patients might be associated with the oncogenic transformation of the gene.

Supported by FAPESP

0763

RENAL IMPAIRMENT IN MULTIPLE MYELOMA PATIENTS FOLLOWING ZOLEDRONIC ACID OR IBANDRONATE TREATMENT

L. Antràs,[1] R. Weide,[2] H. Köppler,[2] M. Smith,[1] M. Neary,[3] J. Green,[3] N. Wintfeld,[3] M.S. Duh[1]

[1]*Analysis Group, Inc, Boston, USA;* [2]*Praxisklinik für Hämatologie und Onkolo, Koblenz, Germany;* [3]*F. Hoffmann-La Roche, Inc, Nutley, NJ, USA*

Backgrounds. Although bisphosphonates prevent skeletal complications, agents differ with respect to renal safety. Ibandronate (IB) is a single-nitrogen, noncyclic bisphosphonate that has shown a renal safety profile comparable to placebo in phase III trials. This retrospective study aimed to compare renal impairment rates in multiple myeloma (MM) patients treated with IB or zoledronic acid (ZO). *Methods.* Medical records in a German oncology clinic from May 2001 to December 2005 were retrospectively reviewed. Creatinine measurements were analyzed from baseline (before ZO or IB treatment) to last evaluation for each patient. Renal impairment was defined as (1) a serum creatinine (SCr) increase of ≥0.5 mg/dL or ≥1.0 mg/dL from baseline values of <1.4 mg/dL or ≥1.4 mg/dL, respectively, or (2) a ≥25% decrease in glomerular filtration rate (GFR; abbreviated MDRD formula) from baseline. Patients treated sequentially with both ZO and IB were included as separate observations. Multivariate analyses were conducted using the Cox proportional hazards model and the Andersen-Gill (A-G) extension of the Cox model for multiple-event analysis. *Results.* In 84 MM patients, 69 received ZO and 40 received IB, with 25 patients receiving both drugs. Compared with IB, the ZO group had a significantly better baseline renal function (mean SCr 1.01 vs 1.34, p=0.006; mean GFR 75.9 vs 57.3, p=0.0002). Data analysis showed that ZO treatment increased the relative risk (RR) of renal impairment by ~3-fold compared with IB (renal impairment rates: ZO 37.7% vs IB 10.8%, RR=3.5, p=0.004 [SCr]; 62.3% vs 24.3%, RR=2.6, p=0.0002 [GFR]). The incidence rate of renal impairment was higher for ZO than IB (SCr: 1.03 vs 0.18 events per person-year, p<0.0001; GFR: 2.93 vs 0.89 events per person-year, p<0.0001). IB

patients who switched from ZO treatment had a significantly higher risk of renal impairment than IB monotherapy patients (renal impairment rates: switchers 40.9% vs monotherapy 6.7%, RR=6.1, p=0.023 [SCr]; 63.6% vs 26.7%, RR=2.4, p=0.029 [GFR]) but experienced a significant trend towards improved renal function during the IB treatment period after a significant trend towards renal deterioration in the ZO treatment period. Multivariate analysis using the Cox proportional hazards model and the A-G model for multiple-event analysis consistently found significantly higher hazards ratios for ZO over IB, after adjusting for differences in characteristics between the two treatment groups. (SCr: Cox=4.2, p=0.016; A-G=8.0, p<0.0001; GFR: Cox=4.2, p=0.001; A-G=3.6, p<0.0001). *Conclusions.* In this retrospective review, MM patients were significantly more likely to experience renal impairment with ZO than with IB. Among IB patients, those previously treated with ZO had a higher risk of renal impairment than monotherapy patients. A prospective randomized study is warranted for further validation.

0764

LOW DOSE BORTEZOMIB, DEXAMETHASONE, THALIDOMIDE PLUS LIPOSOMAL DOXORUBICIN IN RELAPSED AND REFRACTORY MYELOMA

F Leoni, C Casini, C Breschi,[1] R Alterini, C Nozzoli, A Bosi, S.C. Ciolli

Dept. of haematology, Careggi Hospital, Florence, [1] Dept of Oncology, Del Ceppo Hospital, Pistoia, Italy; , Careggi Hospital, Florence, Italy

Background. Bortezomib, a proteasome inhibitor, has proven effective in phase II/III clinical trials. Due to anthracyclines and bortezomib possible interactivity, their combination is attractive. Liposomal doxorubicin has a longer half-life than standard doxorubicin, lower cardiac toxicity and comparable efficacy. It can be safely used in elderly patients or in those with prior exposure to anthracyclines. Thus, we added nonpegylate liposomal doxorubicin (Myocet_) to low dose bortezomib, dexametasone and thalidomide regimen (LD-VTD) we previously tested in R/R myeloma. *Aims.* evaluate the feasibility of treatment, ORR and TTP in comparison with LD-VTD. *Methods*. pts were enrolled once they had a measurable disease, irrespective of PS. Planned therapy: bortezomib 1.0 mg/m^2 i.v. bolus days 1, 4, 8 and 11 of a 28-d cycle, Myocet μ50 mg/m^2 i.v. on day 4, oral dexamethasone 24 mg on the day of and the day following each bortezomib dose. Thalidomide 100 mg/d if non controindicated. Response was defined according EBMT criteria. Patients with PD were removed from the study, the others continued until best response for a maximum of 4 cycles. The time to response was from the date of the first administration of bortezomib to the first evidence of response. *Results.* 20 pts, median age 64 yrs, entered the study: 4 stage IIA,14 IIIA and 2 IIIB, β2microglobulin >4 mg/L in 8 (40%). PS >2 in 7 pts and grade 2 PN in 8. Nine (45%) were primary refractory and 11 R/R. Median time from diagnosis was 6 years, A median of 4 (range 2-6) were the prior therapy lines. All pts had previously received thalidomide plus dexametasone and 6 (1 refractory, 5 relapsed) the LD-VTD regimen. At February 28th 2006, 38 cycles (median 2/pts) were delivered. Thirteen (65%) pts did not receive thalidomide due to previous neurotoxicity. Haematological toxicity presented at day 14-17 and lasted for a maximum of 4 days: grade 2 neutropenia in 3 pts, grade 4 thrombocytopenia in 5. One patient thrombocytopenic at study entry, had a grade 4 gastric haemorrhage. Two pts had pneumonia, 2 HZV infection. Other adverse advents of grade >1 were fatigue (50%), nausea (60%), diarrhoea (15%), alopecia (10%). None experienced progression of PN. No case of DVT or cardiac failure was recorded. Except for 3 pt, all were treated on an outpatient basis. Sixteen pts are valuable for response: 2 were removed from the study for progression, one had stable disease. Thirteen responders : 1 CR, 3 nCR, 8 PR,1 MR (ORR 81%). Median time to best response 1,2 months (range 1- 3). After a median follow-up of 7 months, 19 patients are alive. Conclusions. toxicity was acceptable and most pts were treated on an outpatient basis. Cytopenias recovered within the rest period. A significant neurological toxicity was not recorded, probably due to the lower dose of bortezomib applied. Although an adequate follow-up is needed to draw any conclusion about TTP and OS, this regimen appears very effective as we achieved an ORR of 81% vs 53% previously attained (*Ciolli et al,2006*).

0765

ANALYSIS OF RESPONSE AND FOLLOW-UP IN RELAPSED REFRACTORY MYELOMA RECEIVING BORTEZOMIB

A. Rubio-Martnez, V. Recasns, P. Delgado, J.C. Garca-Zueco, D. Rubio-Flix, P. Giraldo

Miguel Servet University Hospital, Zaragoza, Spain

Background. Bortezomib, a boronic acid dipeptide a novel targeted therapy is a proteaosome inhibitor that has been shown to be effective in the therapy of multiple myeloma (MM). *Aims*. To evaluate the efficacy of Bortezomib in patients with refractory relapsed MM treated in our Department from December 03 to January 06. Patients and Methods. We have included 34 patients with relapsed MM that had been treated with one or more lines of therapy, (VBCMP/VBAD, VAD, PBSCT one or tandem, TACYDEX, radiotherapy). Bortezomib (1,3 mg/m^2 twice weekly two weeks on days 1,4,8,11 in a 21-day cycle) in an outstanding regime were administrated. The response was evaluated according to the criteria of the Consensus Report Scientific Advisor International Myeloma Foundation 2003. CR: without symptoms, no monoclonal component (MC) detected by immunofixation electrophoresis (IFE) (Sebia standardized procedure), PR: reduction of MC >50%, Minimal Response(MR): reduction of MC 25-50%, Clinical response (Clin R): no clinical symptoms, and non-response(NR). Adverse effects were registered. Results. 34 patients (males 41.7%), mean age 60 years (35-74), over 65 years (50%). Type IgG: 11 (6 II-A, 5 III-A); IgA: 12 (7 II-A, 5 III-A); BJ: 7 (3 II-A, 4 III-A), BJ+IgG: 2 II-A, 1 III-A, BJ+IgA: 1 IIIA. Previous therapy: 1 schedule: 5 (15.4%), 2: 10(30.7%), 3: 17(50%), 4: 1(3.8%). For the analysis 26 patients were valuables. Response were reached in 76.9%: (CR+PR 65.4%), (CR 46.1%), (CR-IFE negative 23.0%), (PR 19.2%), (Clin R 11.5%). Mean courses to reached response: 3.6. No relation to response and presence or not chromosomal aberrations were observed. At 24 months on follow-up 7 patients had dead (20.6%) and 11 (42.3%) maintained response without therapy. In 11 patients (42.3%) a combination of Bortezomib+Dexa or Melphalan were administrated by relapse or progression. *Adverse events*. Thrombocytopenia (grade 3: 5, grade 1: 1) 46.1%, fatigue 5 (38.5%), peripheral neuropathy 4 (30.8%), constipation 3 (23%), diarrhea 2 (15.4%), ZHV 2 (15.4%), pneumonia 2 (15.4%), pyrexia 1 (7.8%), hypotension 2 (15.4%), grade 3 leucopenia 1 (7.8%). In 2 patients (15.4%) the therapy was disrupted by toxicity. *Conclusions.* Bortezomib in monotherapy induce a high rate of response (76.9%) in refractory MM. The response is achieved in the first 4 courses. It is recommendable to make combinations after the 4th course of Bortezomib if response does not achieved. No severe adverse effects have been observed with an incidence of reversible haematological side effects in 46.1% and mild non-haematological side effects in 92%.

0766

TUMOR-HOST INTERACTIONS IN THE BONE MARROW OF MULTIPLE MYELOMA PATIENTS: ANALYSIS OF CLINICAL RELEVANCE AND PROGNOSTIC SIGNIFICANCE

J. Bila, M. Perunicic, I. Elezovic, M. Bakrac, N. Suvajdzic, M. Gotic, D. Tomin, A. Vidovic, V. Cemerikic, D. Boskovic

Institute of Hematology, Belgrade, Serbia and Montenegro

Complex pathogenesis of multiple myeloma (MM) includes a functional interplay between the myeloma cells and microenvironment resulting with the interaction of various cytokines, their receptors and adhesion molecules. The aimof study was to analyze prognostic significance of different tumor-host interactions in the bone marrow (BM) of MM patients (pts) by immunohistochemical markers of angiogenesis, osteoclastogenesis and sensitivity to the IL-6. The study included 60 newly diagnosed MM pts (33 male and 27 female pts, mean age 60 years, range 35-75). According to the clinical stage (CS, Salmon&Durie), distribution of MM pts was as follows: I 8pts, II 22pts, III 30pts. Renal impairment existed in 17pts. There were 35pts with IgG monoclonal (M) protein, 12pts with IgA, and 12pts with secretion of kappa/lambda light chain. None secretory MM was diagnosed in lpts. Regarding IPI score, the group included 18pts in stage1, 13pts in stage2, and 29pts with IPI 3. All patients were treated with conventional chemotherapy. In order to analyze microvessel density (MVD), BM vessels were visualized by immunohistochemical staining for CD34. The number of vessels per 400x high power field (HPF) was counted in the area of the most dense vascularization. All samples were further analyzed for the immunohistochemical expression of FGFR-3, OPG, RANKL, and gp130. The intensity of these stainings was graded as weak (0-30% positiveness), moderate (31-60% positiveness), and strong (>60% positiveness). Control specimens were obtained from pts without hematological malignancy.

MVD was significantly higher in MM pts in III CS than in the pts in I CS (15 vs.7,5/ x400 field, p<0,001). Similar finding was obtained by the comparison of pts with IPI 3 and IPI 1(17,5 vs. 9,7/ x400 field, p<0,05). The expression of FGFR-3 was found significantly higher in III CS than in I CS (47,5 vs. 25%, p<0,05), and in pts with IPI 3 comparing to the pts with IPI 1 (60 vs. 22,5%, p<0,001). Significantly strong expression of RANKL was detected in III CS and in pts with IPI 3, comparing to the pts in I CS (67,5 vs. 38,5%, p<0,05) and pts with IPI 1 (55 vs. 38,5%, p<0,05). This correlated with low expression of OPG in III CS (Me 27,5%, range 10-40%), and in pts with IPI 3 (Me 20%, range 5-30%). Also, strong expression of RANKL correlated with the number of recorded skeletal events (20/25 pts). Analysis of the expression of gp130 indicated higher expression in III CS than in I CS (32 vs.15%, p<0,05). Strong activity of angiogenesis and osteoclastogenesis in III CS indicated significantly shorter overall survival of those pts in comparison to the pts in I CS (26 vs. 43,5m, log rank, p<0,05). Similarly, the overall survival of pts with IPI 3 was significantly shorter comparing to the pts with IPI 1 (19,5 vs. 36m, log rank, p<0,001). The assessment of the activity of angiogenesis, osteoclastogenesis and sensitivity to the growth cytokines represents important predictive factor with strong clinical relevance in terms of prognosis of myeloma and application of the various novel therapeutic strategies.

0767

EPIGENETIC MODIFICATIONS OF THE DLK1/GTL2 IMPRINTED GENES IN MULTIPLE MYELOMA AND WALDENSTROMS MACROGLODULINEMIA; PRELIMINARY RESULTS

L. Benetatos,[1] A. Dasoula,[2] E. Hatzimichael,[1] I. Georgiou,[#] A. Vassou,[1] M. Syrrou,[2] K. Bourantas[1]

[1]*Medical School, University Hospital of I, Ioannina, Greece;* [2]*Laboratory of General Biology, Ioannina, Greece;* [3]*Laboratory of Genetics, Medical School, Ioannina, Greece*

Background. *DLK1* and *GTL2* are imprinted genes on human chromosome 14q32. *DLK1* belongs to the Notch epidermal growth factor-like family of receptors and ligands, is paternally expressed and encodes a cell surface transmembrane protein, while GTL2 is maternally expressed and encodes a non-translated protein. Loss of imprinting (LOI) is an epigenetic error associated with tumorigenesis giving the neoplastic clone a growth advantage. *Aims*. The aim of the study was to investigate whether abnormalities of the differentially methylated region (DMR) of *GTL2* promoter have a pathogenetic role in multiple myeloma (MM) and Waldenstrom's macroglobulinemia (WM). Methods. We have studied 9 newly diagnosed patients (7 MM- 2 WM), 5 male and 4 female, with median age 66.3 years (range 49-82). In the subset of the MM patients 1 (14.3%) presented plasma cell leukemia, 1 (14.3%) had indolent MM, 2 (28.6%) had stage II disease, and the resting 3 (42.8) had stage III disease. Cytogenetic analysis was performed only in 2 of the patients, and the karyotypes were apparently normal. DNA methylation pattern was determined by methylation-specific PCR of samples previously subjected to bisulphite-treatment, according to preestablished procedures. Subjects who have undergone bone marrow aspiration for diagnosis of thrombocytopenia, and after we had excluded hematological malignancies served as controls. *Results*. DNA methylation status of the deriving from both blood and bone marrow cells was evaluated. The normal pattern consists of 2 bands (alleles), namely one corresponding to the methylated paternal allele, (size 160 bp) and one corresponding to the unmethylated maternal allele (size 120bp).We found that alterations of the DMR were present in 5 (55.55%) of the patients, of which 1 (11.11%) had WM while 4 (44.44%) had MM. In the patient with the indolent MM we have detected the methylation abnormal pattern in both bone marrow and peripheral blood while in the remaining 4 patients the abnormal methylation pattern was detected only in peripheral blood samples (absence of the unmethylated allele). No association was observed between disease stage and methylation status. *Summary/conclusions*. A total of 18 samples were studied and in 6 (33.33%) we have found alteration of the methylation pattern. It is probable that LOI through epigenetic modifications in the DMR of the GTL2 gene represents a potential pathogenetic mechanism in MM and WM. This is an ongoing study. We will study a larger number of patients in order to verify our preliminary findings. Moreover we will try to find if there is any correlation between disease stage and epigenetic alterations of these imprinted genes.

0768

A MULTICENTER RETROSPECTIVE ANALYSIS OF ADVERSE EVENTS IN KOREAN PATIENTS USING BORTEZOMIB FOR MULTIPLE MYELOMA

S. Bang,[1] J. H. Lee[1] S. Park,[2] C. Kim,[3] S. K. Sohn,[4] K. Kim,[5] H. Yoon,[6] C.S. Kim,[7] Y.S. Kim,[8] H.J. Shin,[9] J.H. Won,[10] G.W. Lee,[11] H. Kim,[12] H.Y. Kim,[10] J.H. Kwon[14]

[1]*Gil Medical Center, Incheon, South-Korea;* [2]*Seoul Nat. Univ. College of Medicine, Seoul, South-Korea;* [3]*Catholic HSCT Center, Seoul, South-Korea;* [4]*Kyungpook National University Hospital, Daegu, South-Korea;* [5]*Sungkyunkwan Univ. School of Medicine, Seoul, South-Korea;* [6]*Kyunghee University Hospital, Seoul, South-Korea;* [7]*Inha University Hospital, Incheon, South-Korea;* [8]*Kosin University Gospel Hospital, Pusan, South-Korea;* [9]*Pusan Nat. Univ. College of Medicine, Pusan, South-Korea;* [10]*Soon Chun Hyang University Hospital, Seoul, South-Korea;* [11]*Gyeong-Sang Nat. Univ. College of Med., Jinju, South-Korea;* [12]*Ulsan University Hospital, Ulsan, South-Korea;* [13]*Wonju College of Medicine, Wonju, South-Korea;* [14]*Kangdong Sacred Heart Hospital, Seoul, South-Korea*

Backgrounds. The proteasome inhibitor bortezomib has demonstrated clinical activity in patients with multiple myeloma (MM). Adverse events including thrombocytopenia and peripheral neuropathy affected 30% to 60% of patients overall, and interrupted therapy in 10% to 20%. There is no prior toxicity data available for Asian patients using bortezomib for MM. Aims. To evaluate the pattern of adverse events in patients treated with bortezomib for their MM. *Methods.* We reviewed the clinical records of patients with the diagnosis of MM from 25 centers in Korea using the NCI Common Toxicity Criteria version 3.0. The included patients were treated with bortezomib alone or in combination with other agents including thalidomide. Results. Ninety-five patients with MM were treated; patients had a median age of 60 years (range: 42-77). The median number of previous treatments was 3 (range: 0-10), 39% of patients had been treated with four or more major classes of agents including thalidomide (67%) and autologous stem cell transplantation (51%). Regimens included bortezomib only in 38 (40%), bortezomib plus dexamethasone in 34 (36%), and bortezomib plus a thalidomide-containing regimen in 23 (24%) patients. The analysis of patient response to therapy revealed: CR + nCR in 31 (33%) and PR in 30 (32%), for an ORR of 65% in 93 patients. The most common adverse events reported were thrombocytopenia (47%), sensory neuropathy (42%), anemia and leukopenia (both 31%). Thirteen patients (14%) stopped therapy due to adverse events; neuropathy in 8, infection in 4 and diarrhea in 1 patient. A neuropathy, more than grade 2 , was more frequent in patients who received 4 or more prior therapy regimens (17/37) compared to those receiving 3 or less (14/58). Also combination of thalidomide was significantly correlated with neuropathy of grade 1~3 (p=0.001). We identified six therapy-related deaths (6%) within 20 days after the last dose of bortezomib. Causes of death were infection in 3, disease progression in 2 and suicide in 1 patient. *Conclusions*. The incidence of thrombocytopenia and neurotoxicity were similar, however gastrointestinal toxicities were relatively low in Korean patients compared to toxicities reported in western studies. Significant neuropathy was associated with the number of prior regimens and combination with thalidomide. These findings provide useful information for clinicians and patients using bortezomib.

0769

FREQUENCY AND DISTRIBUTION OF TRISOMY 11 IN MULTIPLE MYELOMA PATIENTS : RELATION WITH OVEREXPRESSION OF CCND1 GENE AND T(11;14) TRANSLOCATION

T. Guglielmelli, T. Guglielmelli, E. Giugliano, S. Cappia, M. Papotti, G. Saglio

Univ. of Turin and St Luigi Hospital, Orbassano, Italy

Backgrounds. CCND1 is an established oncogene located on chromosome band 11q13 for which genomic rearrangement or amplification leading to overexpression of the cyclin D1 protein is commonly found as a clonal lesion in many human cancer. In mantle cell lymphomas cyclin D1 protein is present in almost all cases and is activated by the characteristic t(11;14) translocation. Also 50% of human breast cancers exhibit cyclin D1 protein overexpression: in 20% of these tumours amplification of the 11q13 region is present but in the remaining cases overexpression cannot be explained by increased gene copy numbers, suggesting that pathogenic activation of cyclin D1 can occur via additional mechanisms. In carcinomas (including colon, lung, oesophagus,

head and neck) and melanomas, cyclin D1 is activated by gene amplification and is associated with poor prognosis. CCND1 overexpression have also been found in 25-50% of multiple myeloma (MM) cases. A molecular classification of MM, named TC classification, stratifies patients into five groups (TC1-TC5) based on the presence of the recurrent IgH chromosomal translocations and cyclins D expression. Patients overexpressing CCND1 can be divided into two groups: TC1, characterized by the t(11;14) or t(6;14) translocation with overexpression of CCND1 or CCND3 and a non hyperdiploid status and TC2, with low to moderate levels of CCND1, absence of any primary IGH translocation and a hyperdiploid status. *Aims*. To assess CCND1 gene and cyclin D1 protein overexpression in a series of primary MM patients, to explore its relationship to the presence of the t(11;14), and to evaluate frequency and distribution of trisomy 11 in the different TC groups. Methods. fluorescence *in situ* hybridization (FISH) analysis with specific probes for CCND1 gene amplification (probe mixture of cyclin D1 band 11q13 - CEP 11 bands 11p11-q11) and t(11;14)(q13;q32) were performed on CD138-purified plasmacells from bone marrows of thirty MM patients at diagnosis. Cyclin D1 protein expression and intensity was evaluate by immunohistochemistry. *Results.* FISH analysis revealed CCND1 overexpression in 14/30 cases (46.6%) and the presence of the t(11;14) translocation in 9/30 cases (30%) (Table 1). Patients with evidence of the t(11;14) showed strong nuclear staining for cyclin D1 (TC1 group) and 8 out 9 demonstrated CCND1 overexpression. The remaining 6 out 15 cases with increased CCND1 gene copy numbers lacked the t(11;14) and showed low to negative levels of cyclin D1 protein (TC2 group). Globally, the frequency of trisomy 11 was 40% (12/30 patients). It was demonstrated in 3 out 9 cases carrying the t(11;14) (TC1), 5 out 6 overexpressing CCND1 without the translocation (TC2) and 4 out 15 negative for both alterations (TC3-TC5). *Conclusion.* In our data, trisomy 11 don't seems to cause directly overexpression of CCND1 as it is present in 4/15 patients without overexpression of CCND1 and in 3/9 patients carrying the t(11;14). One patient belonging to the TC2 group, overexpresses CCND1 and lacks both trisomy and translocation suggesting that cyclin D1 can be dysregulated by additional mechanisms. In TC2 group trisomy 11 probably may be considered as a recurrent polysomy of the hyperdiploid status.

Table 1.

Case	Age	Sex	MC	FISH* CCND1	FISH t(11;14)	Trisomy	IHC§ Cyclin D1
1	58	F	Gk	(3-4)	t(11;14)	–	++++
2	57	M	Gk	(3-5)	t(11;14)	+11	++++
3	72	F	Ak	(3-4)	t(11;14)	+11	++++
4	49	F	k	(3-7)	t(11;14)	–	++++
5	71	M	l	(3-4)	t(11;14)	–	+++
6	80	F	Gk	–	t(11;14)	–	+++
7	60	F	Gk	(3)	t(11;14)	–	+++
8	68	M	Ak	(3-4)	t(11;14)	+11	+++
9	66	M	Al	(3-4)	t(11;14)	–	+++
10	57	F	Ak	(3)	–	+11	+
11	62	M	Gk	(3)	–	+11	+
12	40	M	Gk	(3-4)	–	+11	+
13	70	M	Gl	(3)	–	+11	++
14	60	M	Al	(3-4)	–	+11	–
15	64	F	Gk	(3-5)	–	–	–
16	56	M	l	–	–	–	++
17 63	M	Al	–	–	–	–	
18	74	F	Al	–	–	+11	–
19	62	M	Al	–	–	–	–
20	72	F	Gk	–	–	–	–
21	65	F	Ak	–	NP	+11	–
22	61	M	Gk	–	–	–	–
23	63	F	Ak	–	–	+11	–
24	64	M	Gl	–	NP	–	–
25	63	F	l	–	–	–	–
26	71	M	Gk	–	–	–	–
27	73	F	Gk	–	–	–	–
28	54	M	Ak	–	–	–	–
29	72	F	Gl	–	–	–	–
78	M	Gl	–	–	–	–	–

**In the presence of CCND1 overexpression, the number of copies for each gene is indicated. NP: not performed; MC: monoclonal component. §IHC score ++++ >75% tumor cell positive; +++ 50-75% tumor cells positive, 25-50% tumor cells positive, +10-25% tumor cells positive.*

0770

RAPID DETECTION OF RESPONSE TO BORTEZOMIBE-BASED REGIMEN IN MULTIPLE MYELOMA USING FREE LIGHT CHAIN ASSAYS

R.H. Hajek,[1] P. Pour,[2] H. Novotna,[2] Z. Cermakova,[2] Z. Adam,[2] M. Krejci,[2] K. Havlikova[2]

[1]*Faculty Hospital Brno, Brno, Czech Republic;* [2]*Faculty Hospital, Brno, Czech Republic*

Background. Immunoassays measuring free light chains (FLC) in serum are useful for diagnosis and monitoring of multiple myeloma (MM). FLC assay is also perspective tool as early indicator of response due to short half-life of light chains. This feature would be helpful in the clinical decision of therapy continuation. Bortezomibe (Velcade) represents powerful anti-myeloma agent with rapid clearance of monoclonal immunoglobulin (M-Ig). More than half, respectively 83% responding patients (pts.) achieved first response after second cycle, respectively after cycle 4 in APEX trial. The benefit of FLC assay in detection of responders and non-responders to bortezomibe based regimens has never been evaluated. *Aims*. To evaluate the possibility of using the FLC assay as early marker of sensitivity or resistance to bortezomibe based regimens. *Methods.* Patients with at least one relapse of MM were prospectively evaluated on day 1 and 11 of every cycle of bortezomibe based regimen during the course of their treatment. The sensitivities of serum FLC assays (lambda or kappa, index l/k) and M-Ig analysis using immunoelectrophoresis for detection of early response were compared in 3 categories: time to reduction of parameters to 25% (MR), 50% (PR) and 75% of the pretreatment value. Data of 24 patients from total of 37 pts. who underwent at least 5 cycles of therapy with median 7 (5-8) cycles were chosen for pilot analysis. *Results*. Total of 13 pts. (54%) responded to the therapy with 4% of CR, 29% of NCR (only immunofixation poz.), 21% of PR. Further 17% pts. achieved minimal response and 25% of pts. (6/24) had stable disease. The significant difference was found in the time of response detection: PR (>50%) was achieved on day 22/44/66 of the treatment in 41.7%/54.2%/54.2% (single chain), in 16.7%/16.7/33.3 (index λ/κ) and in 12.5%/37.5/54.2 (M-Ig). The difference was statistically significant when comparing single chain vs. M-Ig on day 22 (p=0.026), on day 44 (p=0.005) but not on day 66 and not when comparing the index l/k vs. M-Ig. Direct comparison of timing of response (time to reduction to 75%, 50% and 25% of pretreatment value) was done for well-correlated parameters single chains and M-Ig. Difference at the first level of response (MR - reduction to 75%) was not found (median 16.6 vs. 22 days; p=0.424) but the results were significantly better for single chains at the level of PR [50% reduction; median 16.5 (range 11-44) vs. median 44.0 (range 22-111); p<0.001] and at the level of 75% reduction. [Median 11.0 (range 11-55) vs. median 66.0 (range 33-88); p=0.054]. We did not observe sustained PR in pts. who had not responded before day 33 if FLC assay was used. *Summary/Conclusions*. In the pivotal analysis we have confirmed that FLC assays is perspective tool for detection of early responding pts. with MM treated with bortezomibe based regimens. On the contrary, the FLC assay has potential to be used as a marker of early resistance. The trial is under way and actual results will be presented.

Funding: Supported by LC06027 MSMT .

0771

BORTEZOMIB THERAPY IN MULTIPLE MYELOMA EXPERIENCE OUTSIDE THE CONTEXT OF A CLINICAL TRIAL

S. Sachanas, M.C. Kyrtsonis, Z. Galanis, V. Greca, T.P. Vassilakopoulos, K. Anargyrou, S. Chrysochoou, M. Dimou, G. Panagoulias, S.I. Kokoris, E.M. Dimitriadou, M.K. Angelopoulou, N. Viniou, P. Panayiotidis, G.A. Pangalis

University of Athens, Athens, Greece

Backgrounds. Bortezomib (Velcade™) reversibly and selectively inhibits the proteasome and degrades primarily ubiquitinated proteins. Bortezomib produces high response rates in MM, as shown by two large clinical trials. Based on these results, the drug was approved for the treatment of relapsed and refractory multiple myeloma as a third or second line therapy. *Aims.* To present our experience with Bortezomib treatment in relapsed and refractory MM patients treated according to approved indications, outside the context of a clinical trial. Patients and Methods. 52 MM patients (38 males and 14 females) with a median age of 70 years were treated with Bortezomib. Immunoglobulin type was IgG in 30 (2 with secondary plasma cell leukemia), IgA in 15, BJ in 6 and IgD in 1. 36 patients had already received 2 treatment lines while 14 one. 46% had been previously treated with thalidomide At treatment ini-

tiation none of the patients had peripheral neuropathy > grade 1. Patients were scheduled to receive bortezomib 1.3 mg/m^2 IV (days 1, 4, 8 and 11) every three weeks for eight cycles and dexamethasone was planned to be added at a dose of 20 mg every other day (days 2, 5, 9, 12), if no signs of response were observed after the 1st cycle. Actually, 27 patients received dexamethasone and the 2 patients with plasma cell leukemia received thalidomide in addition. *Results*. Two patients in terminal resistant disease died prematurely and 3 patients are still receiving the 2nd cycle (thus, 5 patients are not evaluable at present). Overall, 39 out of 47 evaluable patients responded (83%) (3 complete remission [CR], 5 near CR, 25 partial response, 6 minimal response). The median time to response was 42 days. Within a median follow-up of 8 months (2-160), 16 (34%) patients relapsed and 8 patients (15%) died, 7 of disease and 1 of unrelated cause. In the majority of patients, non-neurologic toxicity was mild and reversible including fever, fatigue, gastrointestinal symptoms and hematologic toxicity. The most severe side effect was peripheral neuropathy which developed in 60% of patients. Neuropathy included ataxia, caustic pain and sensory disturbances and resolved after a median time of two months after discontinuation of Bortezomib. In most patients, treatment was reduced or stopped because of peripheral neuropathy but all responding patients completed at least 4 cycles. *Conclusion*. Bortezomib therapy alone or in combination with low dose dexamethasone produces rapid responses in relapsed and refractory MM. Early relapses are frequent. Neuropathy is the most important adverse reaction and lead to dose reduction or discontinuation of treatment.

0772

BORTEZOMIB AS A SINGLE AGENT IN REFRACTORY/RELAPSED MULTIPLE MYELOMA RESULTS OF CZECH MYELOMA GROUP (CMG)

I.S. Spicka

General Fac. Hosp. Prague, Prague, Czech Republic

Summary. The efficacy of single agent bortezomib in the treatment of refractory multiple myeloma has been shown repeatedly. We summarize the results of bortezomib therapy in Czech republic. 82 patients with a median age 61 years (33-84) with refractory/relapsed myeloma were scheduled to receive Velcade 1.3 mg/m^2, on days 1,4, 8 and 11 mostly of a 21-day cycle. This was a heavily pretreated population (1-8 prior therapies, median 2), including high-dose therapy with stem cells support (56 patients, 68%) and/or thalidomide (42 pts, 51%). The overall results, assigned by EIBMT criteria, were as follows: the response was achieved in 40 patients (48.8%) - eight patients had complete (9%), 19 partial (29%) and 8 minor (9%) response. In 10 cases stabilization of disease was observed, 20 patients progressed during therapy, 5 died early after the start of therapy and in 7 cases the evaluation was not available due to short time of therapy. The response was observed early after the start of therapy in most cases, in 33 patients after the first cycle (40%) and in 11 after the second cycle (13%), although in minority of them progression during further therapy was observed. The most common adverse events were thrombocytopenia (65,9%) and neuropathy (52,4%), however, grade 3/4 thrombocytopenia developed in 37,8% and neuropathy only in 7,3% of patients, respectively. Other grade 3/4 complications were anemia (6%), granulocytopenia (13%), gastrointestinal events (8,5%), renal failure (6%) and infections (5%). *Conclusion*. Our experience confirmed that bortezomib provides clinical benefit with manageable toxicities in this heavily pretreated and high-risk population.

0773

SYNERGISTIC GROWTH INHIBITION OF MALIGNANT PLASMA CELLS BY DEXAMETHASONE AND THE FARNESYLTRANSFERASE INHIBITOR L744.832

A. Ott, F. Bakker, K. Richter, T. Ahrens, R. Burger, A. Guenther, M. Gramatzki

2*nd Medical Department, Kiel, Germany*

Background/Aim. In this study, we assessed the in vitro efficacy of combinations of conventional and novel drugs on human multiple myeloma cell lines. Aim of this study was to test whether novel agents are able to enhance the response of malignant plasma cells towards conventional therapeutics. This may provide rationale for combination therapies which could improve the outcome of multiple myeloma. *Methods*. Cell lines JK-6L and L363 were incubated with various concentrations of drugs, alone or in combination, in the presence or absence of human bone marrow stromal cells (BMSC). Cell growth was measured in an MTS assay and results were evaluated for synergism. *Results*. It was found that dexamethasone (Dex) and the farnesyltransferase inhibitor L744.832 (L744) inhibited the growth of both cell lines in a synergistic fashion (e.g. 0.4 μM Dex reduced the growth of JK-6L cells to 91% of that observed in untreated controls, 0.4 μM L744 reduced growth to 38% of untreated controls, combining both agents reduced growth to 24%). Interestingly, L744 was able to restore Dex sensitivity of Dex resistant cells (JK-6L in the presence or absence of BMSC, L363 in the presence of BMSC) as well as to further enhance the sensitivity of Dex sensitive cells (L363 in the absence of BMSC). Furthermore, the rate of apoptosis, upon treatment of the cells with either agent alone or in combination, was determined by Annexin V (AxV) staining. Combining both agents enhanced the cytoxicity of either drug alone (e.g. in JK-6L cells, 0.1 μM Dex led to a 4.8% increase in apoptotic rate compared to untreated controls, 0.25 μM L744 led to a 10.7% increase, combining both agents led to a 21.4% increase), confirming the data obtained from the cell growth assays. *Summary/conclusions*. L744 synergizes with Dex and is able to enhance and restore the Dex sensitivity of malignant plasma cell lines. This in vitro study provides rationale to explore the use of this combination of agents in patients with multiple myeloma.

Chronic lymphocytic leukemia and related disorders Clinical/Experimental II

0774

DEXAMETHASONE INDUCES APOPTOSIS *EX VIVO* IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS WITH EITHER UNMUTATED IGVH GENES OR HIGH ZAP-70 EXPRESSION

A. Muntaola, M. Crespo, E. Gine, N. Villamor, E. Montserrat, F. Bosch

Hospital Clinic, Barcelona, Spain

Background. Patients with chronic lymphocytic leukemia (CLL) and unmutated IgVH genes or high ZAP-70 expression have poorer prognosis than those with mutated IgVH genes or low ZAP-70 levels. This is in part related to the resistance of unmutated and ZAP-70 positive cases to treatment agents that induce apoptosis in a p53-dependent manner. It has been suggested that corticosteroids are active in CLL through p53-independent pathways. *Aims.* To analyze the *ex vivo* response to dexamethasone in CLL cells according to the IgVH mutational status and ZAP-70 levels, and the expression of different glucocorticoid receptors in CLL cells. *Methods*. Frozen lymphocytes from 60 patients with CLL were analyzed for ZAP-70 expression and IgVH mutational status (n=44). Cells were cultured and treated using Fludarabine (5 μgr/mL), Mitoxantrone (0.5 μgr/mL), FCM (fludara 1 μgr/mç, maphosphamide 1 μgr/mL and mito 0.5 μgr/mL), FM (fludara 1 μgr/mL and mito 0.5 μgr/mL), Dexamethasone (5.2 μgr/mL) and FMD (fludara 1 μgr/mL, mito 0.5 μgr/mL and dexa 5.2 μgr/mL). Cell viability and apoptosis were determined by annexin/PI staining and FACscan analysis at three different time points and conditions (0 h and 24 h without treatment, and 24h with each treatment). The expression of glucocorticoid receptor (GR) isoforms α, β and γ was analyzed by Quantitative RT-PCR in 20 cases. *Results*. Dexamethasone-induced apoptosis was significantly higher in samples with unmutated IgVH genes and/or high ZAP-70 expression (≥ 20%) than in those with mutated IgVH genes and/or low ZAP-70 expression (median cell viability 65% vs. 81%, respectively; $p<0.001$). In contrast, the highest cell mortality induced by mitoxantrone was observed in samples with IgVH mutations or low ZAP-70 expression ($p=0.009$). Median cell viability was 56.1% for FM vs. 34.3% for FMD ($p<0.0001$) regardless of the IgVH mutational status. No differences in cell viability were found according to ZAP-70 expression or IgVH mutational status after *ex vivo* treatment with fludarabine or FCM ($p=0.649$ and $p=0.055$, respectively). No relationship was found among IgVH mutational status and the expression of the different GR isoforms. Expression of the three different GR isoforms was also similar in corticosteroids responders and non-responders. *Conclusions.* In this study, CLL cells with unmutated IgVH genes or high ZAP-70 expression showed a higher cell mortality after *ex vivo* exposure to dexamethasone than those with mutated IgVH genes or low ZAP-70 expression, with no relationship with the expression of the different GR isoforms. These data give conceptual support to trials aimed at determining the role of dexamethasone in the treatment of patients with CLL and poor prognostic features or resistant to fludarabine.

0775

CLL IN RADIATION EXPOSED AFTER CHERNOBYL: IMMUNOPHENOTYPIC AND PATHOLOGICAL FINDINGS

D. Bazyka, D. Bazyka, O. Polyschuk, N. Belyaeva, I. Ilyenko, I. Kryachok, V. Bebeshko

Research Center for Radiation Medicine, KYIV, Ukraine

Background. CLL becomes an intriguing issue in radiation medicine. After Chernobyl a significant excess of CLL rates was demonstrated in radiation exposed contrary to previous studies considering it not to be radiation-induced. *Aims*. The aim of this study was to find possible CLL differences in radiation exposed. *Methods*. CLL clinical course, blood and bone marrow cells morphology, immunophenotype, histology of BM were analyzed in 80 former Chernobyl radiation recovery workers with doses range from 10 to 1180 mSv and 70 unexposed male patients. Results. CLL in exposed was diagnosed at earlier age (mean age 55.6±0.8 in comparison with 62.4±0.9 in unexposed),was characterized by the shorter period of WBC doubling (10.7 vs 18.0; $p< 0.001$) frequent infectious episodes, lymphoadenopathy and hepatosplenomegaly (37 vs 16). CLL/PLL morphology and phenotypic features were more frequent in the first group of patients. Higher expression was demonstrated for CD5, CD20, CD38, CD23 HLADR and lower for ZAP-70 antigens. No differences were demonstrated by biodosimetry in the TCR-4$^+$ variant cell counts. The diffuse type of lymphocytic infiltration in BM of the majority of exposed was associated with clinical course in exposed patients but no differences were shown in quantitative parameters by morphometry. Study of *in vitro* cytokines production has shown the decrease of spontaneous IL-2, γ-INF production and an increase of IL-1, IL-4, IL-10, TNF synthesis. *Conclusion*: Presented data demonstrate the presence of certain differences related presumably to cell activation and humoral factors production. That could explain peculiarities of the clinical course. No explanation was obtained of the increased CLL rates in radiation exposed.

0776

CYTOSOLIC HISTONE H1.2 RELEASING UNDER DIFFERENT APOPTOTIC STIMULI IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

E. Gin, M. Crespo, A. Muntaola, E. Montserrat, F. Bosch

Hospital Clinic, Barcelona, Spain

Background. Recently, it has been shown that nuclear histone H1.2 is released into cytoplasm when apoptosis is induced by DNA double-strand breaks (DSB's), this process being dependent on p53 functional status. In addition, cytosolic histone H1.2 induces cytochrome C release in a Bak-dependent manner. Thus, cytosolic histone H1.2 release represents a new mechanism that links DSB's with activation of the apoptotic mitochondrial pathway. Aims. Against this background, we analyzed the release of histone H1.2 in the cytosol of purified CLL cells during apoptosis induced by fludarabine (F), mitoxantrone (M), etoposide, dexamethasone and ionizing radiation. In addition, the presence of histone H1 was correlated with p53 functional status. *Methods*. Cell viability and apoptosis were investigated by annexin V/PI staining and FACscan analysis. The presence of histone H1 and H1.2 in the cytosolic fraction was assessed by Western Blott using the anti-histone H1 (Upstate) and anti-histone H1.2 (Abcam) antibodies. Histone H1 traffic was also evaluated by using immunofluorescence analysis in CLL cells suspensions. FISH analysis was used to select samples with (n=7) or without (n=10) p53 deletion, and activation of p53 after treatment was assessed by Western Blot. Results. In cases without p53 deletion, increased apoptosis was observed under all stimuli, the FM combination being the most effective. In such cases, histone H1.2 release was apparent 6 hours after the onset of irradiation or pharmacologic treatments, progressively increasing up to 24 hours. In contrast, cases with p53 deletion displayed a low cytotoxic effect upon different treatments. Interestingly, no p53 activation or histone H1.2 release into cytosol was observed in those cases with a high p53 deletion level (>79%, n=4,). Moreover, DXM which induces apoptosis in a p53 independent manner was the only treatment tested able to induce the cytosol appearance of histone H1.2 in three of these cases These results were also confirmed by immunofluorescence analysis, in which histone H1.2 was only visible in the cytosol of non-deleted p53 cases. *Conclusions*. These results demonstrate that, upon drug or irradiation exposure nuclear histone H1.2 is released into the cytoplasm of CLL cells in a p53-dependent manner. This suggests that, in CLL, histone H1.2 traffic contributes to the apoptosis induced by DSB´s and to drug resistance in cases with p53 deletion.

0777

ZAP-70 EXPRESSION IN NEOPLASTIC CELLS AND T LYMPHOCYTES OF B-CLL PATIENTS: A REPRODUCIBLE METHOD FOR DETECTION USING FLOW CYTOMETRY

J. Caetano, M. Laiges, M. Gomes da Silva, T. Faria, M. Jorge, A. Parreira, P. Lúcio

Instituto Portugus de Oncologia, Lisboa, Portugal

Background. Prognostic stratification in B-CLL is critical to design informative therapeutic trials. The best combination of biological and clinical parameters for patient classification in prognostic groups should include Zap-70 expression, which correlates with lack of somatic mutations of immunoglobulin V-H genes. Determination of Zap-70 expression by flow cytometry is attractive due to wide accessibility and speed, but is hampered by lack of standardization. *Aim*. The aim of this study was to identify the best strategy for flow cytometric analysis of Zap-70 expression in B-CLL cells, and to compare it with other prognostic factors. Methods. ZAP-70 expression was determined in PB samples of 39 patients (19 males, 20 females; median age 63.3, 31-85 years), including 23 stage A and 16 stage B or C. Zap-70 expression was determined within 24 h of sample drawing. In 18 patients the determination was performed before treatment. Anti-CD19, CD20, CD22, CD23, FMC7, CD79b, CD38, CD3, CD5, Zap-70 (PE-labeled), K and L fluorescent monoclonal antibodies were used, and a minimum of 5000 events were acquired. We determined the percentage of B-CLL cells expressing Zap-70 with intensity equal or higher than T cells. If Zap-70 was expressed in more than 20%, tumor cells were considered positive. To reduce inter-observer and technical variability, we further calculated the ratio between the median fluorescent intensity (MFI) for ZAP-70 in B-CLL and T cells. The relationship with CD38 expression (positive if present in more than 30% of neoplastic cells) and genetic abnormalities (delection of 13q, 11q, 17p and, trisomy 12, evaluated by FISH) was determined using the χ^2 test. Time-to-first-treatment was calculated using the Kaplan-Mayer method and the influence of Zap-70 expression evaluated by the log-rank test. *Results*. With a median follow-up of 34,7 (0-145) months only one patient died. Median time-to-first-treatment was 11,3 (0-132,9) months, with 7 patients receiving fludarabine-based regimens, 12 alkylating agents, and 2 antracyclines (global response rate 62%). Patients were divided in 3 groups according to the presence of the following cytogenetic findings: del17p and/or del11q (20,5%), trisomy 12 or no abnormalities (38,5%), and del13q (23,1%). Seventy four percent were Zap-70 positive, while only 36% were CD38 positive. Zap-70 positivity was unrelated to Binet stage, CD38 expression and cytogenetic findings (p=0,235). Time-to-first-treatment was similar in Zap-70 positive and negative patients (p=0,99). However, when the ratio between MFI of Zap-70 in B-CLL and T cells was used, we found that patients with ratios higher than 0,4 had a prolonged time-to-first-treatment as compared to patients with lower ratios (p=0,03). *Conclusions*. Determination of Zap-70 expression using the ratio between Zap-70 MFI in tumor and T cells is a reproducible method for Zap-70 evaluation in B-CLL, by simultaneously providing an internal control for the fluorescence intensity of positive cells and reducing the inter-observer and technical variability.

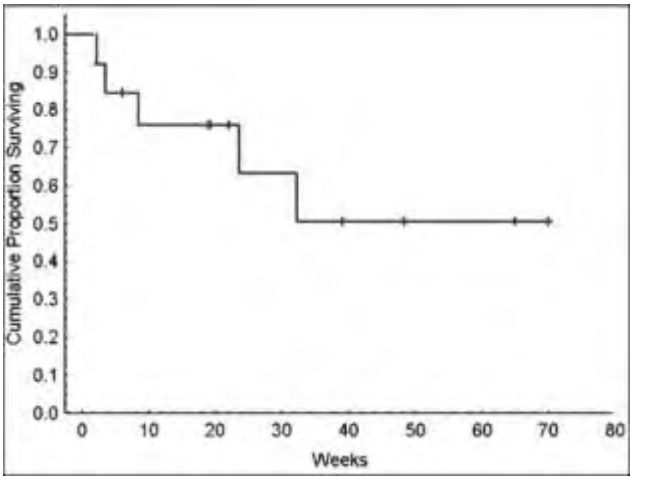
0778

A PHASE II STUDY OF THE COMBINATION OF ALEMTUZUMAB AND PENTOSTATIN IN PATIENTS WITH T-LYMPHOID MALIGNANCIES

F. Ravandi, H. Kantarjian, S. Faderl, S. Verstovsek, C. Koller, W. Wierda, A. Ferrajoli, F. Giles, M. Keating, S. O'Brien

University of Texas, MD Anderson, Houston, Texas, USA

Backgrounds. Mature T-cell lymphoid neoplasms are clonal disorders of post-thymic T-cells that are considered separately from precursor T-cell leukemias. These neoplasms are generally refractory to traditional chemotherapy regimens and as such, the prognosis for most patients is poor; novel therapeutic strategies are needed. Alemtuzumab and pentostatin have reported response rates of 50% to 75% when used individually, but most responses are partial or of limited duration. Immunosuppression and risk of opportunistic infections are their principal overlapping toxicities. *Aims.* To determine the feasibility and efficacy of the combination of alemtuzumab and pentostatin for the treatment of T-lymphoid malignancies. *Methods.* We have treated 13 patients with T-lymphoid malignancies (7 T-PLL, 1 ATL, 1 T-ALL, 2 γ/δ-T cell hepatosplenic lymphoma, 2 T-LGL) with a combination of alemtuzumab 30 mg IV three times weekly for up to 3 months and pentostatin 4 mg/m^2 weekly x 4 followed by alternate weekly administration for up to 6 months. Prophylactic antibiotics including valacyclovir, trimethoprim/sulfamethoxazole, and fluconazole (or equivalents) were administered during the treatment and for 2 months after completion. *Results.* The median age of patients was 57 years (range 22-80 years), median white blood count was 60.5×10^9/L (range 0.6-279.5×10^9/L), median serum β-2M was 4.1 mg/L (range, 2.2 to 10.8 mg/L). Four patients had splenomegaly (1-6 cm), and 5 lymphadenopathy. Eight had prior therapy (median 3, range 1 to 6 regimens). Eight patients have responded (7 CR, 1PR) for an overall response rate of 62%. Monoclonal T-cell receptor chain gene rearrangements were detected by PCR in 7 patients and became negative in 2 of 4 evaluable patients in CR. Median response duration is 5+ months (range 0.25+ to 13+ months).



Two patients have proceeded to allogeneic stem cell transplant, 2 (1 with ATL and 1 with T-LGL) have died from disease progression after an initial response, and 5 were refractory to therapy or died early from disease-related complications. Opportunistic infections have included reactivation of CMV in 3 patients, reactivation of HSV in 1 patient, recurrence of pre-existing *Serratia pneumonia* in 1 patient and Aspergillus pneumonia in 1 patient. Other toxicities were mainly grade 1 and 2 and included nausea, fever, fatigue, infusion reactions, edema, and shortness of breath. *Conclusions.* The combination of alemtuzumab and pentostatin is feasible and effective in T-cell neoplasms. Although infections including CMV reactivation are a concern, they may be minimized with adequate prophylactic antibiotic therapy and close monitoring of patients. Accrual is continuing.

0779

WNT5B EXPRESSION: A NEW POTENTIAL PROGNOSTIC MARKER IN CHRONIC LYMPHOCYTIC LEUKEMIA

C. Rabascio, L. Saronni, V. Raia, P. Mancuso, A. Calleri, C. Massaro, P. Antoniotti, J. Quarna, D. Laszlo, G. Martinelli, F. Bertolini

European Institute of Oncology, Milan, Italy

Background. ZAP70 and CD38 expression, along with heavy chain (IgH) mutational status, are currently under investigation as predictive markers in chronic lymphocytic leukemia (CLL). Previous data (Desheng et al PNAS 2004) suggested that Wnt signaling pathway contributes to a deficient apoptosis in CLL. Wnt5b is a ligand for members of the frizzled family of seven transmembrane receptors. It may be a signaling molecule which affects the development of some regions of tissues. Correlation of ZAP70, CD38 expression and IgH status with Wnt5b expression. *Method.* This retrospective study was conducted on 14 newly diagnosed and 9 relapsed CLL patients and on 18 healthy donors. We used the ABI PRISM 7000 Real Time platform to analyze Wnt5b expression, which was normalized against healthy donors median expression (P/H). We also evaluated CD38 (Ibrahim et al Blood 2001), ZAP70 expression (Crespo et al N Engl J Med 2003) and IgH rearrangement (Theriault et al Mod Pathol 2000) as previously described. *Results.* All healthy donors and 15 CLL patients showed Wnt5b expression, while 8 CLL patients were negative. After a median follow-up of 21,5 months (range 8-29), 6 patients were in complete remission, 11 were in stable disease (SD) and 6 had progressive disease (PD). 14/15 patients (93%) with Wnt5b expression at diagnosis were in PD or SD (p=0.01 vs patients in CR) at the end of follow-up. All patients who achieved a CR after therapy had no or very low Wnt5b expression at diagnosis (medi-

an P/H=0, range 0-0.26), while SD or PD patients had a median P/H ratio higher than 0.3 (range 0-10.52, p=0.01). ZAP70 was evaluated on 20/23 patients. 15 of them were ZAP70+ (75%) and 5 were ZAP70- (25%). 9 patients were ZAP70+/Wnt5b+; 88% of them had a SD or PD; 3 patients ZAP70-/Wnt5b+ showed no CR. Among the 6 patients ZAP70+/Wnt5b- 3 (50%) obtained a CR. Both patients showing ZAP70-/Wnt5b- achieved a CR. 21 patients were also evaluated for CD38 expression; 62% (13/21) were CD38- and 70% of them were Wnt5b+, 38% were CD38+ and 50% of them were Wnt5b-. IgH status was evaluated in 12 patients before and after treatment; 9 of them (75%) maintained a monoclonality and 3 (25%) became polyclonal. 67% of monoclonal patients at revaluation were Wnt5b+ at diagnosis, 84% of them achieved PD or SD, while 33% of polyclonal patients were originally Wnt5b+. Conclusion: In our CLL population, Wnt5b diagnosis expression suggests an association with poor outcome. Among ZAP70- patients (good prognosis) Wnt5b expression seems to further select patients with a worse prognosis. We are prospectively evaluating a larger patient population to confirm the predictive potential role of this novel biomarker in a multivariate analysis. The availability of these novel biologic prognostic indicators might be of relevance for future risk-adapted treatments.

0780

T CELL SUBSETS AND CMV INFECTION IN CLL: ASSOCIATIONS WITH IGVH MUTATIONAL STATUS, GENE USAGE AND CHROMOSOMAL ABERRATIONS

H.D. Alexander, C. Matthews, M.A. Catherwood, T.C.M. Morris

Belfast City Hospital Trust, Belfast, Northern Ireland

Background. Chronic lymphocytic leukaemia (CLL) is characterised by a profound dysregulation of the host's immune system. This results in recurrent infections and an increased incidence of autoimmune diseases, suggesting T-cell dysfunction. Furthermore, the frequent use of potentially more immunosuppressive therapies such as fludarabine and alemtuzumab, has also increased the incidence of recurrent infections, such as cytomegalovirus (CMV), in CLL. CMV is a member of the herpes family of DNA viruses and arguably causes the most morbidity and mortality of any herpes virus. CMV establishes life-long latent infection without clinical disease in immunocompetent individuals, but can cause severe illness in newborns, transplant recipients, cancer and HIV patients. *Aims.* This study was designed to determine the frequency of CMV DNAemia in CLL patients, in comparison to normal age and gender matched controls. Associations were sought between evidence of CMV DNAemia and elevated T-lymphocyte subset numbers to determine if T-subset expansions could be used as surrogate markers of CMV infection. IgVH mutational status, VH gene usage and chromosomal aberrations were determined to ascertain if CMV DNAemia occurred more frequently in patients with markers of poor outcome. Methods CMV DNA copy number was measured using RQ-PCR CMV kit on a light cycler. IgVH mutational status and VH gene usage were determined using BIOMED-2 primers and protocol, while the presence of adverse cytogenetics was ascertained using interphase FISH technique. Results CMV DNA was detected in 95/113 (84%) of CLL patients. Six of these had DNA copy number > 1×10^5, while 18/113 (16%) had no detectable viral DNA. CMV infection was significantly more common in CLL than normal age matched controls. Additionally, the presence of elevated CMV DNA copy numbers was not dependent on prior exposure to chemotherapeutic agents. No significant associations between CMV DNAemia and T cell subsets or markers of poor prognosis were noted. However, significant associations between high absolute cytotoxic T cell (CTLs) counts and poor prognosis chromosomal aberrations, and advanced clinical stage (Binet B&C), were recorded (p=0.017, p=0.026, respectively). Intriguingly, patients with VH3-21 rearrangements were found to have high CMV titres (p=0.049), whilst VH3-7 gene usage was linked to lower absolute T cytotoxic counts (p=0.009). *Conclusions.* This study demonstrated that T cell subsets could not act as surrogate markers for CMV DNAemia. Significant $CD8^+$ T cell expansions were identified in the cohort of patients with poor prognosis cytogenetic abnormalities and advanced clinical stage. T cell dysfunction in CLL has been suggested to contribute to the aetiology and progression of the disease by being unable to start, maintain and complete an immune response to the malignant B cell and other antigens. Additionally T cells have been shown to produce cytokines that prevent CLL cells from entering apoptosis. This may lead to the accumulation of cytogenetic aberrations and therefore may be involved indirectly in sustaining the tumour. Finally, the intriguing finding of high CMV DNA copy numbers in patients with VH3-21 rearrangements suggests that CMV infection may play a role in the poor outcomes in these patients.

0781

SERUM THYMIDINE KINASE LEVELS CAN IDENTIFY EARLY STAGE B-CLL PATIENTS WITH MUTATED IGVH GENES MOST LIKELY TO PROGRESS

H.D. Alexander, C. Matthews, M.A. Catherwood, T.C.M. Morris

Belfast City Hospital Trust, Belfast, Northern Ireland

Backgrounds. Thymidine kinase (TK) is a cellular enzyme which functions as part of the pyrimidine salvage pathway involved in DNA synthesis. Serum TK levels have been shown to be correlated with survival in many malignancies, including CLL. *Aims.* This study was designed to investigate associations between TK levels and other prognostic markers, in newly diagnosed Binet stage A patients. Furthermore, the use of serum TK measurement to identify subcategories of disease within those defined by IgVH mutational status, gene usage, CD38 expression, Zap-70 positivity and chromosomal aberrations, was studied. *Methods.* Ninety-one CLL patients were recruited to this study. Serum TK levels were measured using a radioenzyme assay. IgVH mutational status and VH gene usage were determined using BIOMED-2 primers and protocol. Recurring chromosomal abnormalities were detected by interphase fluorescent in-situ hybridisation (FISH). CD38 and Zap-70 expression were determined by flow cytometry and RT-PCR respectively. *Results.* The 91 patients (53 male, 38 female) had an age range of 37-94 years, median 71 years. Forty-five of the 91 patients were newly diagnosed Binet stage A patients, 31 were previously diagnosed early stage patients and 15 cases had advanced CLL (Binet C/Rai III/IV). Significantly higher serum TK levels were found in IgVH unmutated, compared to IgVH mutated, patients (p<0.001). Elevated TK levels were also associated with CD38 positivity (p=0.013), short LDT (p=0.026) and poor and intermediate prognosis chromosomal aberrations (p<0.001). A TK level of greater than 8.5 U/L, best identified patients with progressive disease. Within the newly diagnosed group, nine IgVH mutated cases had a TK level of 8.5 U/L, or greater. Closer scrutiny revealed that these patients had either VH3-21 or VH1-69 gene usage and/or had short LDT of <12 mths, which are associated with poorer outcomes. Additionally, within the unmutated subgroup of newly diagnosed patients, only one had a TK level lower than 8.5 U/L. This particular patient had not undergone lymphocyte doubling, greater than four years after diagnosis, which was longer than that seen in the remaining unmutated cases, with the highest TK values recorded in patients with shorter lymphocyte doubling times. *Conclusions.* Unlike IgVH mutational status, TK does not lose predictive power as disease progresses, with the highest TK levels reported in advanced clinical stage. This study demonstrated that determining serum TK level at diagnosis in early stage patients can identify those most likely to progress and therefore require earlier treatment. Furthermore, the variations in disease progression within prognostic subcategories can be predicted by measuring serum TK levels at diagnosis, allowing further refinement of risk stratification. We confirm the efficacy of TK measurement in CLL to determine proliferation activity and predict clinical course of this heterogeneous disease.

0782

DIAGNOSTIC POTENTIAL OF CD38 COMBINED WITH ZAP-70 EXPRESSION IN PREDICTING MUTATIONAL STATUS OF IMMUNOGLOBULIN HEAVY-CHAIN VARIABLE REGION IN 450 CHRONIC LYMPHOCYTIC LEUKEMIA CASES

F. Morabito,[1] G. Cutrona,[2] M. Gentile,[1] S. Matis,[2] E. Vigna,[1] S. Molinu,[2] M. Colombo,[2] M. Spriano,[3] E. Rossi,[3] G. Festini,[4] C. Gentile,[1] C. Stelitano,[1] S. Molica,[5] S. Zupo,[2] V. Callea,[1] M. Ferrarini[2]

[1]Hematology Unit, COSENZA, Italy; [2]Natl. Institute for Cancer Res, GENOA, Italy; [3]ASL S Martino, GENOA, Italy, [4]SL Trieste, TRIESTE, Italy; [5]ASL Pugliese-Ciaccio, CATANZARO, Italy

Backgrounds. Recent advances in the diagnosis and molecular characterization of CLL permit improved prediction of disease prognosis, which could result in better management. The best-studied parameters are somatic hypermutation of the immunoglobulin heavy-chain variable region (VH), expression of the cellular proteins CD38 and ζ-associated protein 70 (ZAP-70). *Aims.* In this study, we analyzed the mutational status of the VH genes in CLL cells from a series of 450 cases and correlated the results with CD38 expression detected by flow-cytometry and ZAP-70 using Western blotting. *Methods.* Determination of Zap-70 was carried out by Western blot. For the purpose of this study, samples showing a negative and weak ZAP-70 patterns were collectively analysed. The B-CLL Ig VH gene usage and mutation was determined on cDNA according previously reported methods. The best cut-off point for CD38 or Zap-70 expression for discriminating between mutated and unmutated IgVH status was sought by constructing receiver operating

characteristic (ROC) curves, which were generated by calculating the sensitivities and specificities of data at several predetermined cut-off points. Moreover, the usefulness of CD38 and ZAP expression in identifying VH mutational status was analysed according to the following standard diagnostic tests: sensitivity and specificity, positive and negative predicted values and accuracy, as well as by Kappa statistic. *Results.* As a first step, we determined, by ROC curve analysis, 30% as the best cut-off value of CD38 which discriminates between mutated and unmutated cases in CLLs (area under the curve 0.758, $p<0.0001$). On the basis of standard diagnostic tests, CD38 expression, categorized by 30% cut-off value, had relatively low sensitivity (70%), specificity (77%), negative predictive (76%) and positive predictive (71%) values in anticipating VH mutational status. Moreover, Kappa statistic revealed that the agreement between CD38 expression and VH mutational status was low although significant (K=0.47, $p<0.001$). On the other hand, ZAP-70 showed very low sensitivity (57%), high specificity (89%), low positive predictive value (57%), relatively low negative predictive value (72%) and a low, although significant, K statistic (0.47, $p<0.001$). Furthermore, we combined the value of both tests to evaluate whether both variables provided more precise information in estimating VH mutational status compared to that obtained from each single test . In this regard, we obtained the following *Results.* sensitivity, 90%; specificity, 96%; positive predictive value, 90%; negative predictive value, 76%; k statistic 0.43, $p<0.001$). Moreover, ROC analysis was also performed to detect the optimal percentage of Ig V gene mutations capable of predicting cases with positivity of both CD38 and Zap-70. The best cut-off value was 1.9% (AUC 0.814, $p<0.0001$), which is close to the threshold (2%) used to distinguish mutated from unmutated B-CLL. *Conclusion/Summary*. Our data demonstrated that neither CD38 nor ZAP-70 by themselves had an important impact in anticipating VH mutational status. When CD38 and Zap-70 were combined, the predicting model significantly improved, meaning that the combined use of CD38 and Zap-70 could surrogate the prognostic value of VH mutational status. This information should be validated on clinical ground.

0783

VH3-48 AND VH3-53 GENE REARRANGEMENTS REPRESENT UNIQUE SUBGROUPS IN CLL AND ARE ASSOCIATED WITH BIASED λ LIGHT CHAIN RESTRICTION, HOMOGENOUS LCDR3 SEQUENCES AND POOR PROGNOSIS

H.D. Alexander, C. Matthews, M.A. Catherwood, T.C.M. Morris

Belfast City Hospital Trust, Belfast, Northern Ireland

Backgrounds. In recent years IgVH mutational status, VH gene usage, and the potential role of antigens in the leukomogenesis of chronic lymphocytic leukaemia (CLL), have been studied extensively. In particular, the identification of VH3-21 gene usage as a unique subset of CLL has lead to questioning of the prognostic limitations of IgVH mutational status, as VH3-21 usage is associated with poor prognosis, irrespective of the fact that two thirds of such patients have mutated IgVH genes. Furthermore, this specific gene has been linked with highly homogeneous heavy and light complementarity determining regions (CDR3), indicating that these patients possess virtually identical BCR binding sites and thus suggesting a common antigenic progenitor. *Aims.* The aims of this study were to scrutinize VH gene usage in a large cohort of CLL patients to determine if other VH gene rearrangements could identify similar prognostically significant subgroups of patients. Furthermore, we sought to identify the frequency of VH3-21 rearrangements in a Northern Irish population of CLL patients, and determine if chromosomal aberrations associated with poor outcomes could account for the poor prognosis in these patients. Methods. Two hundred and twenty eight patients were recruited from Belfast City Hospital Haematology Outpatient Clinic and surrounding regional hospitals. Clinical staging (Rai and Binet), immunophenotyping, lymphocyte doubling time (LDT) and time to treatment (TTT) were available on all patients. IgVH and IgVL mutational status, gene usage and CDR3 sequences were determined using multiplex BIOMED-2 primers and protocol and sequence analysis. FISH analysis was performed on all patients. *Results.* VH3-21 gene usage (n=18) was associated with poor prognosis, overuse of VL3-21 (Vλ2-14) gene and highly homogenous heavy and light CDR3 sequences. Only one VH3-21 patient showed an adverse prognosis chromosomal aberration. VH3-48 (n=8) and VH3-53 (n=4) gene rearrangements showed biased lambda light chain restriction (7λ: 1κ, 3λ: 1κ, respectively). Further analysis revealed overuse of VL3-21 (Vλ2-14) gene (7/8) and highly homogenous LCDR3 sequence (*QVWDSSSDHPWV*) in VH3-48 patients. Both VH3-48 and VH3-53 categories showed a preponderance of females, short LDT (<1-21 months) and an absence of any poor prognosis chromosomal aberrations. *Conclusions.* This study showed that the incidence of VH3-21 gene usage in Northern Ireland (7.9%) lies between that reported in Scandinavian (12%) and Mediterranean populations (0-3%). Furthermore, VH3-48 rearrangements, together with VH3-21 and possibly VH3-53, represent unique subgroups in CLL associated with poor prognosis, irrespective of mutational status. The recurrent use of specific VL genes and homogenous LCDR3 sequences in these patients suggests a common aetiological factor.

0784

IDENTIFICATION OF NEW GENOMIC ALTERATIONS IN CLL USING A 32K BAC CGH ARRAY

R. Gunnarsson,[1] J. Staaf,[2] G. Jönsson,[2] K. Karlsson,[2] K. Willander,[3] A. Borg,[2] G. Juliusson[4]

[1]*Lund University, Lund, Sweden;* [2]*Department of Oncology, Lund University, Lund Sweden;* [3]*Department of Hematology, Lund University Hospital, Sweden;* [4]*Stem cell center, Lund University, Lund, Sweden*

Backgrounds. Cytogenetics in CLL is hampered by low mitotic index. Genetic screening for prognostic factors has been routinely performed by FISH for established abnormalities. All of them are unbalanced, and some (e.g., del13q14) are believed to be early events of pathogenesis, whereas others (del17p) may be associated with progression of disease. To get a more detailed profile of genomic alterations in CLL we applied a genome wide arrayCGH. *Aims.* To screen the genome of CLL cells using a high resolution 32 K BAC array. To map and delineate established and novel genomic alterations, with a view to prognostic and pathogenic ramifications. To correlate the genomic data with IgVH mutation status and outcome for patients in the Scandinavian phase III study with primary/initial treatment for symptomatic CLL. *Methods.* Genomic DNA from 74 pretreated patients was hybridized to a 32K BAC array produced by Swegene DNA Microarray Resource Center, Department of Oncology, Lund University, Sweden (*http://swegene.onk.lu.se*). The array covered the human genome with a tiling resolution of ~100 Kbp. The array data was analyzed with BioArray Software Environment (*http://base.thep.lu.se/*). *Results.* Preliminary analysis of the DNA copy number profiles allowed detection of previously described genomic changes along with identification of novel alterations. The most common alteration among the CLL samples was del13q14 (40%) followed by del11q22 (30%). In 13 out of 28 samples displaying the del13q14 homozygous deletion was implied. The minimal deleted region (MDR) could be mapped to a region of 0.1 Mb encoding the genes DLEU1, DLEU2 and DLEU7. Losses of chromosome 11q spanned from 11q14.1 to 11q23.2 with the peak at 11q22.3. Trisomy 12 was detected in 23% of the samples, in several samples indicating only partial gains. Loss of the 17p arm was also detected, in some cases with a concurrent gain of the 17q arm. Genomic changes were detected in the majority of samples. For example, losses on 2q36, 5q13.2.12, 18q21.2 were detected in individual samples. Recurrent gains were mapped to 6p21.3 and 8q21.2. *Summary/Conclusions.* The high resolution CGH array combines full genome screening with high specificity and allows detection of small lesions. Genomic abnormalities were identified in most patients, including the well-known changes, and can be delineated accurately. Validation of novel changes and correlation studies will follow, and may improve our understanding of genomic lesions and their clinical implications in CLL.

0785

EVALUATION OF TRANSFERRIN RECEPTOR 1 AND 2 EXPRESSION AT THE RNA AND PROTEIN LEVEL IN NORMAL B CELLS VS. CHRONIC LYMPHOCYTIC LEUKEMIA

I. Chiotoglou,[1] M. Samara,[1] T. Smilevska,[2] K. Stamatopoulos,[2] C. Belessi,[3] S. Lykousi,[1] N. Stavroyianni,[2] G. Paterakis,[4] N. Stathakis,[1] N. Laoutaris,[3] A. Fassas,[2] A. Anagnostopoulos,[2] P. Kollia[1]

[1]*University of Thessaly Medical School, Larissa, Greece;* [2]*G. Papanicolaou Hospital, Thessaloniki, Greece;* [3]*Nikea General Hospital, Piraeus, Greece;* [4]*G. Gennimatas Hospital, Athens, Greece*

Transferrin receptor 1 (TfR1, CD71) is one of the classical activation markers up-regulated upon B-cell activation. TfR2 has significant sequence homology with TfR1 and also binds transferrin, albeit with lower affinity than TfR1. The TfR2 gene has two alternatively spliced transcripts (α and β): in normal tissues, TfR2-α mRNA is more abundant than TfR2-β mRNA. Chronic lymphocytic leukemia (CLL) is characterized by almost ubiquitous CD71 expression (*Smilevska et al., Leuk Res. 2006;30:183-9*); this is in keeping with the activated status of CLL cells. In the present study, we evaluated TfR1 and TfR2 expression at the mRNA and protein level in 76 CLL patients as well as $CD19^+$ B cells

sorted from peripheral blood (PB) mononuclear cells of two healthy donors. In all CLL cases, the tumor load ($CD5^+CD19^+$ cells) was at least 70%. Forty-eight out of 76 CLL cases (63%) carried IGHV genes with <98% homology to the closest germline gene (mutated); the remainder (28/76 cases, 37%) carried unmutated IGHV genes. TfR1 and TfR2 cDNA sequences were detected by RT-PCR in PB CD19+ B cells from both healthy donors. All CLL samples were tested positive for TfR1 cDNA sequences; TfR2-α/β cDNA sequences were detected by RT-PCR in, respectively, 39/76 and 70/76 CLL cases. Real-time PCR (using h-HPRT as a housekeeping gene) revealed comparable TfR1 mRNA expression in $CD19^+$ B cells of both healthy individuals (average TfR1/h-HPRT ratios: 0.995) as well as higher TfR2-α than TfR2-β mRNA levels (average TfR2-α/h-HPRT or TfR2-β/HPRT ratios: 0.07 and 0.016, respectively). TfR1 mRNA levels were widely divergent in CLL samples [median TfR1/h-HPRT ratio: 1,3 (0.37-13.3)]. TfR2-α mRNA levels were not significantly different in CLL vs. normal $CD19^+$ B cells [median TfR2-α/h-HPRT ratio: 0.07 (0.008-1.9)]. In contrast, significantly higher TfR2-β mRNA levels were observed in CLL vs. normal B cells [median TfR2-β/h-HPRT ratio: 0.06 (0.005-0.233)]. In CLL, no statistically significant associations were identified between TfR1 or TfR2 mRNA levels and IGH mutation status. TfR1 and TfR2 protein expression was studied by Western blotting with appropriate antibodies. Quantitative gel-banding densitometry was conducted on Epson GT-8000 Laser Scanner. TfR1 protein expression did not differ significantly in normal $CD19^+$ cells vs. CLL (although CLL samples showed greater individual variation in TfR1 band optical density). Faint TfR2 bands were detected in normal $CD19^+$B cells (average optical density: 0.01), in marked contrast to CLL [median optical density: 0.4 (0.15-1.35)]. These results indicate that post-transcriptional mechanisms are of greater importance in regulation of TfR1 expression in malignant CLL vs. normal B cells. TfR2-β mRNA and TfR2 protein expression are significantly more abundant in CLL, suggesting a possible functional role for TfR2 in malignant cells, perhaps other than iron uptake. Finally, the results of the present study support the notion that the *standard model* of iron homoeostasis, mediated mainly by TfR1 and perhaps by TfR2, remains the main mechanism to meet the demands for iron in either normal or CLL malignant B cells.

0786

NEW PATHWAYS INVOLVED IN APOPTOSIS INHIBITON IN CHRONIC LYMPHOCYTIC LEUKEMIA

R. Proto-Siqueira

Faculty of Medicine of Ribeirao Preto, Ribeirao Preto, Brazil

Background. Apoptosis reduction mediated mainly by increased expression of BCL2 is a major mechanism of accumulation of mature B lymphocytes in peripheral blood (PB) and bone marrow in chronic lymphocytic leukemia (CLL). The disease comprises two subtypes that are characterized by important outcome differences observed between patients expressing either mutated (MUT) or unmutated (NAÏVE) immunoglobulin genes. *Aims.* We analyzed the gene expression of the malignant cells of CLL (MUT and NAÏVE) to identify possible additional molecular mechanisms related to decreased apoptosis. Materials and methods. $CD19^+$ lymphocytes were isolated by positive magnetic cell sorting (MACS) from six CLL patients (3 MUT and 3 NAÏVE), three mantle cell lymphoma (MCL) patients, and 4 normal naïve B-lymphocytes isolated from tonsils. Gene expression profiles were obtained by serial analysis of gene expression (SAGE) and individual gene expressions were measured by real time PCR and semi-quantitative RT-PCR. We also evaluated, by real-time PCR, the expression of BCL2, ZAP-70, CFLAR and TOSO (FAIM3) in 22 CLL, 8 MCL patients and 8 normal CD19+ PB cells ($nCD19^+$) obtained from healthy volunteers. Results. Approximately 100,000 tags were sequenced for each CLL subtype, corresponding to 15,000 known genes, whereas for MCL and normal naive B cells we sequenced about over 60,000 tags which matched 16,000-13,000 known genes. The comparison between the two subtypes of CLL reveled only 27 genes with significant ($p< 0.001$) differences. The comparison of the transcriptional profiles from CLL with that of $nCD19^+$ (*obtained from Hashimoto et al, 2003*) revealed that transcripts of ILR4, IL24, TOSO and FMOD were exclusively or highly represented in CLL, whereas JUN, JUN-B, IL-8 and EGR-1, among others, were poorly represented in CLL. Comparison of MCL and naive B lymphocytes revealed that CFLAR was overrepresented in MCL ($p<0.001$). To corroborate SAGE results, observed differences in gene expression levels were validated by semi-quantitative RT-PCR of the same six cases of CLL, 3 cases of MCL, one nCD19+ cells and one normal bulk BM, for 7 different genes: HLADR_5, SIAT-6, SURF-5, ILR4, FMOD, IL24 and IL8. The real-time PCR analysis of 22 CLL, 8 MCL patients and 8 $nCD19^+$ reveals that TOSO were over expressed in CLL cases (CLL x $nCD19^+$ p=0.0130; CLL x MCL p=0.0337). The expression of TOSO correlated significantly with BCL2 and ZAP70 expression in CLL (TOSO x BCL2, Spearman's r=0.5439, p=0.0019; TOSO x ZAP70, r=0.5318 p=0.0025). CFLAR was over-expressed both in CLL and MCL when compared with nCD19+ cells ($p<0.01$). Conclusions. This new approach to evaluate gene expression revealed that both CLL subtypes have very similar profiles. Various differences of the gene expression observed between the CLL and its normal counterpart are related to the deficiency of apoptosis, and among them we demonstrate for the first time the participation of TOSO and CFLAR. They represent addition pathways that contribute to apoptosis inhibition, and the expression of TOSO is probably associated with a poor outcome. Our findings strengthen the role apoptosis deregulation in the genesis of the leukemic phenotype, and reveal novel genes involved in these pathways.

0787

T-CELL TYPE LYMPHOPROLIFERATIVE DISEASE OF GRANULAR LYMPHOCYTES IS EQUIPPED WITH A PHENOTYPIC PATTERN TYPICAL OF EFFECTOR CYTOTOXIC CELLS

R. Zambello, I. Baesso, L. Pavan, E. Boscaro, M. Miorin, M. Facco, N. Maschio, L. Trentin, C. Agostini, G. Semenzato

Padua University School of Medicine, Padua, Italy

In 22 patients with $CD3^+$ LDGL we investigated whether proliferating GL displayed the phenotype of fully differentiated cytotoxic cells. To this aim, according to the recently defined phenotypical pattern recognizing the different stages of cytotoxic effector cell differentiation, we analyzed the expression of CD27, CD28, CD45RA, CD45RO, CD62L, CCR7, IFN-γ on GL surface. Since NK receptors have been found to be central in the pathogenesis of LDGL, we also analyzed the expression of Killer-Immunoglobulin-like Receptors (KIRs), CD94/NKG2, NKG2D and Natural Cytotoxicity Receptors (NCRs) on GLs surface. In all LDGL patients taken into account, GLs were found to be monoclonally rearranged for the T-cell receptor (TCR)γ. In 18/22 patients studied, our data showed that $CD3^+CD16^+$ cells expressed a homogeneous $CD45RA^+$, $CD27^-$, $CD28^-$, $CD62L^-$, $CCR7^-$, INF-γ+ pattern, consistent with those of fully differentiated CTLs. In four cases a coexpression of CD45RA and CD45RO was documented by GL. The majority of these patients (20/22) expressed NKG2D receptor. In addition, KIR receptors were expressed only in a fraction of patients (7/22) as far as CD94/NKG2 (5/22). Interestingly, the activating receptor CD94/NKG2C was detected in 2/5 of $CD94^+$ cases, suggesting that activating signals for cell proliferation might stem from this receptor. In all patients' GLs the NCRs NKp44, NKp46, NKp30 were absent, while NKp80 was expressed in the majority of cases (20/22). In conclusion, our data demonstrated that GLs in $CD3^+$ LDGL patients show a phenotype consistent with that of fully differentiated CTL. The expression of NK receptors, although useful for the definition of diagnosis of LDGL, does not represent a critical feature of the abnormal clone, suggesting that expression of these receptors is indipendent from the acquisition of the in vivo mature CTL phenotype, which indeed represents the truly distinctive phenotypic hallmark in these patients

0788

LOW-DOSE ALEMTUZUMAB MONOTHERAPY IN ADVANCED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

A. Cortelezzi,[1] M.C. Pasquini,[2] G. Reda,[2] F. Ripamonti,[2] B. Sarina,[3] W. Barcellini,[4] G. Lambertenghi Deliliers[2]

[1]*Fondazione Policlinico Università Milano, Milan, Italy;* [2]*University of Study Milan, Milan, Italy;* [3]*Istituto Humanitas, Milan, Italy;* [4]*Ospedale Maggiore Policlinico, Milan, Italy*

Backgrounds. Alemtuzumab is a well established therapeutic tool in CLL and its low-dose (ld) subcutaneous (sc) administration is safe and efficacious. Aim: To confirm on a larger number of patients with a longer follow-up the results of an already published (Cortelezzi A. et al Haematologica 2005, 90: 410-412) pilot study on ld sc alemtuzumab in refractory CLL. *Methods.* Since January 2003 we treated at our Istitutions thirty-six consecutive CLL patients, 33 of whom are evaluable for efficacy. Patient characteristics were as follows: M/F 22/14; median age 68 years, range 48-83; Binet stage C 36.1% , stage B 55.6%, progressive stage A 8.3%; abnormal karyotype 69.4%, including unfavourable alterations (17p- 13.8%, 11q- 22.2%, trisomy 12 19.4% and 6q- 5.5%); ZAP-70 positive at immunoistochemistry55.5%. All the patients were pretrated (median prior lines of therapy 2, range 1-5) and refractory to alkylators, 58.3% were fludarabine-resistant, and 25% also rituximab-resistant. Previous grade 3/4 infections were documented in one third of the patients.

Alemtuzumab schedule was as follows: 10 mg sc X 3/ week for 18 weeks, with anti-infective prophylaxis. Results. The overall response rate (NCIWG criteria) was 48.5%, including 21.2% complete response. Better responses were seen in blood (77.4%), as compared with bone marrow (54.5%), spleen (50%), and lymph nodes (48.5%). Responses were observed in patients with Zap70+ (45%), adverse Karyotype (40%), 17p- (60%), stage C (46%), fludarabine- (47.4%) and rituximab-resistance (33.3%). Progression was documented in 7/16 responders after a median of 12 months (range 6-18 months) After a median follow-up of 15 months (range 1.5-38) the median survival has not been reached for the entire case series (66.6% alive), responders (75%), and non-responders (58.8%). Eleven patients died after a median of 10 months (range 1.5-25) for infectious complications, 2 of them were in remission and 9 in progressive disease. Grade III-IV neutropenia or anemia were recorded in 36.1% and 2.7% of the patients, respectively. Mild anaemia was observed in 55.5% of the patients during alemtuzumab therapy. Coombs-positive AIHA was documented in two patients after the end of Tx administration (9 and 1.5 months), being a reactivation in one of them. Two patients developed ITP one month after stopping alemtuzumab. Infections (1 otitis media due to *Pseudomonas*, 1 dermatomeric *Herpes zoster*, 2 pneumonia, 1 lethal polymicrobial sepsis) occurred during alemtuzumab treatment in 13.8% of the patients. Transient and clinically silent reactivation of cytomegalovirus was documented in 22.2% of the patients by pp65 antigenemia testing or PCR. Eight patients (4 with latent and 4 with reactivated HBV infection) received lamivudine while on alemtuzumab. Adefovir dipivoxil was associated to lamivudine in two cases for an hepatitis flare. These antiviral therapies enables us to complete alemtuzumab Tx in all the patients. *Conclusions.* We confirm on a larger number of high-risk relapsing/refractory CLL patients the high percentage of response, long remission duration, and the favourable toxicity profile of ld sc Alemtuzumab already shown in the pilot study.

0789

ALLOGENEIC STEM CELL TRANSPLANTATION AND CHRONIC LYMPHOCYTIC LEUKEMIA: DISTINCT IMMUNOGLOBULIN VARIABLE HEAVY CHAIN GENES AS A POSSIBLE PROGNOSTIC INDICATOR

I. Panovska-Stavridis, L. Cevreska, M. Ivanovski, L. Hadzi-Pecova, A. Stojanovik, B. Georgievski

Medical Faculty-Skopje, Skopje, Macedonia

Backgrounds. Although the treatment of chronic lymphocytic leukemia (CLL) has changed dramatically over the previous few years, CLL remains an incurable disease. Great proportions of patients relapse early after the treatment and eventually become refractory to treatment. To a number of these subjects allogeneic stem cell transplantation (alloSCT) is being offered, even though the procedure is still considered as experimental approach and is associated with a number of risks. The mutational status of the immunoglobulin variable heavy chain (VH) genes is a major prognostic indicator for the clinical course and outcome of the CLL patients. More recently, additional prognostic categories have been identified by recognizing disease subsets which utilize unique VH genes. Examples include the V3-21, V1-69 and V3-72 genes which are invariably associated with progressive and stable disease, respectively. *Aims.* The aim of our study was to investigate the possibility to identify suitable CLL candidates for alloSCT among CLL subsets that utilize unique VH genes. Methods. The study group consisted of 106 consecutive CLLs that had been diagnosed at our Institution prior to 2000, according to standard morphologic and immunophenotypic criteria. VH gene family usage and mutational status were obtained by direct sequencing of RT-PCR amplified RNA samples. Correlations between the different CLL subsets were made using standard statistical tests. *Results.* Our results showed that 61 (57,6%) patients utilize mutated VH genes, and the rest 45 (42,4%) have unmutated sequence. The most frequently rearranged VH gene in our CLLs was V1-69 gene, in all 25 cases (23.6%) with unmutated sequence. We compared the overall survival (OS) of the V1-69 subgroup against all the other patients. The two groups were comparable regarding the sex, age, total tumor mass (TTM) score and Rai stage. The VH1-69 group has median OS of 56.7 months and all others patients have median OS of 125. 8, (p<0.0001). Then, we further analyzed the differences in survival between VH1-69 cases and all patients with unmutated VH genes. There were no differences between the two subgroups regarding the age, gender, TTM score, Rai stage and OS. No other unique VH gene, utilized in our study group, had frequency important of analyzing. *Conclusion*: Our data do not support the thesis that patients expressing V1-69 gene form a distinct subgroup of CLL patients. Further investigations are needed to reach the definite conclusion regarding the role of V1-69 gene and all others distinct VH genes in the prognosis and treatment decision in CLL patients. Our results confirmed that CLL with unmutated VH gene sequence has poorer OS and we suggest that all younger patients that utilize unmutated VH genes should be consider as candidates for early alloSCT, immediately after the first complete remission, if HLA identical donor is available.

0790

REVERSAL BONE MARROW ANGIOGENESIS AFTER COSOLIDATION THERAPY WITH ALEMTUZUMAB IN ADVANCED CLL

S. Molica,[1] M. Montillo,[2] D. Ribatti,[#] R. Mirabelli,[1] A. Tedeschi,[2] F. Ricci,[2] S. Veronese,[3] A. Vacca,[5] E. Morra[2]

[1]*A.O. Pugliese-Ciaccio, Catanzaro, Italy;* [2]*Niguarda Hospital, Milan, Italy;* [3]*Anatomy Hinstitute, Unversity Bari, Bari, Italy;* [4]*Niguarda Hospital, Milan, Italy;* [5]*University Bari, Bari, Italy*

We have previously shown that in patients with advanced chronic lymphocytic leukaemia (CLL) who respond to therapy with fludarabine bone marrow (BM) angiogenesis decreases significantly. To expand on these observations, we evaluated microvessel area of BM samples from 20 patients with advanced CLL (i.e., symptomatic Binet stage B or C) who received at least 8 weeks after the end of treatment with fludarabine subcutaneous alemtuzumab, three times weekly for 6 weeks, at escalating dose up to 10 mg. The patient sample included 14 males and 6 females with a median age of 51 years (range 44-60). After a median number of 6 cycles of fludarabine (range, 4-13), 11 (55%) patients could be classified in complete remission (CR) and 9 (45%) in partial remission (PR) (7 nodular-PR and 2 PR). Interestingly, the rate of CR increased to 90% (18 CR; P=0.03; Fisher's exact test) after treatment with alemtuzumab. In keeping with hematological responses, significant changes of BM angiogenesis were observed. The assessment of microvessel area carried out at the starting of therapy, after fludarabine and at the end of therapy with alemtuzumab, respectively, showed a continuous decrease in the extent of microvessel area (p=0.002). This conspicuous feature was easily demonstrable in ZAP-70-positive (p=0.02) and ZAP-70-negative (p=0.001) patients. As far as molecular response is concerned, 13 out of 20 (65%) patients changed from a monoclonal to a polyclonal pattern of IgH. A separate evaluation carried out in patients with a persistent monoclonal IgH pattern and in patients who changed to a polyclonal pattern of IgH after therapy with alemtuzumab showed a significant reduction of BM microvessel area only in the latter (p=0.0002). Finally, a significant (p=0.0001) decrease of the extent of BM angiogenesis was observed among patients who received a cumulative dose of alemtuzumab higher than median (i.e.184 mg) while the same did not apply for those who had received cumulative dose of alemtuzumab lower than median (p=0.127). In conclusion, a decrease in BM vascularity was observed after treatment with alemtuzumab. Such a finding reflects either molecular response or cumulative dose of alemtuzumab. These observations lend support to the anti-angiogenic role played by alemtuzumab in CLL.

0791

FLUDARABINE AND CYCLOPHOSPHAMID (FC) VERSUS CYCLOPHOSPHAMIDE, VINCRISTINE AND PREDNISONE (CVP) AS FIRST LINE TREATMENT IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)

T.H. Abdelhamid, N. Al-Lahloby, A. Kamel, N. Elsharkawy, A. Zaher, N. Francis

National Cancer Institute, Cairo, Egypt

Backgrounds. The combination of CVP is known to be active in previously untreated CLL patients. FC is another effective regimen. *Aim.* To evaluate the response rate, time to disease progression and survival of FC (arm A) versus CVP (arm B) as first line treatment. Patients and method: The patients were randomized into the two treatment arms, each 31 patients. The diagnosis of CLL was established according to the criteria of the International Workshop on CLL (IW CLL) 1989. Eligibility criteria were age<65 years, ECOG performance status 0 -II with high risk category (Rai stage III-IV) or Rai stage I-II if they have at least one of the followings: one or more of the disease related symptoms, progressive marrow failure, massive splenomegally or lymphadenopathy or progressive lymphocytosis > 50% in 2 months or lymphocyte doubling time < 6 months. Arm A: Cyclophosphamide 250 mg/m^2 I.V. D1 to D3 and Fludarabine 25 mg/m2 D1 to D3. Arm B: Cyclophosphamide 400 mg/m^2 I.V. D1 to D3, Vincristine 1.4 mg/m2 D1 and Prednisone 100 mg/m^{2}D1 to D5 P.O. Cycles to be repeated every 21 days. Hematological toxicity was recorded according to the NCI sponsored working

group revised guidelines for diagnosis and treatment of CLL (Cheson et al 1996). Evaluation of response was done according to the NCI-WG response criteria. Patient with stable or progressive disease after the 3rd cycle were excluded from the study while PR and CR cases continued to 6 cycles of the same regimen. Bone marrow biopsy and Immunophenotyping were done to confirm the response to treatment. *Results.* The median age for the whole group was 53.5 years (Range 33 -65).They included 42 males and 20 females. Twenty cases had stage III and 21 patients for each of stage II and III. The median TLC was 81×10^9/L.The median lymphocyte count was 70×10^9/L. The median hemoglobin level was 9.2 gm/dl, while the median platelets count was 150×10^9/L. Pretreatment bone biopsy showed diffuse pattern in 49 cases (79%) and the median lymphocyte in bone marrow was 85.5%. Complete clinical remission was reported in 15 /31 cases in arm A (48.4%) compared to 6 /31 in arm B (19.4%) p=0.17. Confirmed CR by bone marrow biopsy was reported in 10 cases in arm A (32.3%) and only 3 cases in arm B (9.7%). Partial response with nodules (PR-nod.) was reported in 5 cases (16.1%) in arm A and 3 cases (9.7%) in arm B. Median time to disease progression was 25 months in arm A and 6 months in arm B (p=0.03). At 2 years, no significant difference in survival between both arms was detected (83.9% for arm A versus 74.2% for arm B). *Conclusion*: The combination of FC is able to induce higher response rate with better quality of response at the level of BM biopsy. There was a statistically significant difference in time to disease progression in favor of FC regimen but no significant difference in overall survival.

0792

ORAL FLUDARABINE AND CYCLOPHOSPHAMIDE IN UNTREATED PATIENTS AFFECTED BY CHRONIC LYMPHOCYTIC LEUKEMIA. A PILOT STUDY

L. Laurenti,[1] A. De Padua,[1] M. Tarnani,[1] P. Piccioni[1], A.M. Wahlstrom,[2] M. Garzia,[1] N. Piccirillo,[1] G. Zini,[1] A. Fiorini[1], G. Farina,[1] C. Rumi,[1] S. Marietti,[1] S. Sica,[1] G. Leone[1]

[1]*Istituto di Ematologia UCSC-Roma, Rome, Italy;* [2]*Sahlgrenska University Hospital, Gothenburg, Sweden*

Backgrounds. The introduction of fludarabine into the treatment of chronic lymphocytic leukemia (CLL) has improved the complete remission rate (CR), overall response rate (OR), and progression free survival (PFS) compared with alkylator based regimens. Its synergistic action with cyclophosphamide has demonstrated significative advantages as front line therapy in untreated CLL patients with advanced disease. The oral formulation of fludarabine showed a similar safety profile and rensponse rate as the endovenous compound. Aims. Primary end-point was to test efficacy and safety of the oral formulation of fludarabine combined with cyclophosphamide as front-line therapy of high-risk CLL. As secondary end-point we examined the impact of new prognostic factors associated with progressive CLL (i.e. unmutated IgVH gene status, positivity for ZAP-70, del(11)(q23), and del(17)(13.1)) on treatment outcome. *Methods.* Starting from December 2002, 30 untreated patients with advanced CLL, 20 male and 10 female, with a median age of 68.5 years (52-75) received oral fludarabine (30 mg/sm) and oral cyclophosphamide (250 mg/sm) for 3 consecutive days every 4 weeks, for a total of 6 cycles. At study entry, 25 patients were in stage B/II with progressive disease, 2 in stage C/III, and 3 in stage C/IV. Twelve patients had unmutated and 11 mutated IgVH genes, while in the remaining 7 patients the IgVH gene mutation status was not evaluable. Fifteen patients had more than 20% ZAP-70 positive CLL-B cells, and four patients had the high rish cytogenetic abnormalities del(11)(q23) or del(17)(13.1). *Results.* Among the 26 evaluable patients, 12 obtained CR (46%), 8 PR (31%). Of the remaining patients 3 had stable disease and 3 progressive disease. In terms of haematological toxicity 6 patients developed grade IV neutropenia and received G-CSF treatment, while two patients developed severe anemia (grade III and IV) that required red blood cell transfusions. Only one patient developed a transient febrile neutropenia of unknown origin, but did not require hospitalization. Mild extra-hematological toxicity consisting of nausea and vomiting occurred in six patients during the treatment. No significant differences were noticed in terms of CR and OR rate between the IgVH mutated and unmutated groups (p=ns). Among the 5 patients who have relapsed so far, 4 had unmutated and only 1 had mutated IgVH genes (p=ns), and all three patients that required new treatment (NCI WG criteria) had unmutated IgVH. *Conclusion*: Oral fludarabine plus cyclophosphamide as front-line therapy in CLL achieved a good overall response rate in our series of patients (46% CR and 31% PR). The haematological and extrahematological side effect were mild and the oral scheme was easy to administer. The differences in terms of CR, OR and PFS between the IgVH mutated and unmutated groups did non reach statistical significance. However, a longer follow-up is required to define the possible correlation between these prognostic factors and treatment outcome.

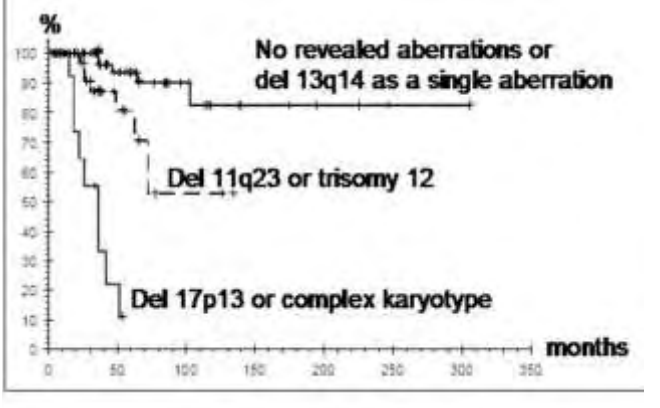
0793

CYTOGENETIC CHARACTERIZATION OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA / B-CELL SMALL LYMPHOCYTIC LYMPHOMA (B-CLL/B-SLL)

A.I. Zakharova, T.N. Obukhova, Y.Y. Lorie, E.V. Domracheva

National Center for Hematology, Moscow, Russian Federation

Background. Chromosome abnormalities studied in a debut of B-CLL/B-SLL provide the important prognostic information. However, inconsistent imformation about the prognostic significance of cytogenetic aberrations in B-CLL/B-SLL is still present. There are also few data available looking in particular at cytogenetic characterization of the rare subtype of this disease - B-SLL. Aims. To define the type and frequency of chromosome changes of B-CLL/B-SLL and to estimate their prognostic significance. *Methods*. We studied 135 patients with B-CLL/B-SLL, 90 men and 45 women. The median age was 58 years (range 23 - 84 years). Median follow-up for survival was 32 months. The diagnosis was made by clinical, cytological, histological and immunofenotypic criteria. CD38 expression was measured by flow cytometry in all patients. IgVH mutation status was determined in 61 patients. We performed conventional cytogenetic assay (CCA) and fluorescence in situ hybridization (FISH) using multicolor probe sets LSI p53 / LSI ATM and LSI D13S319/LSI 13q34/CEP12 on mononuclear cells. All B-CLL patients did not receive specific therapy till the moment of cytogenetic analysis. *Results*. Del13q14 was the most frequent cytogenetic abnormality; it was revealed in 34 (25%) cases. Del11q23 was found in 26 (19%) cases, trisomy 12-in 17 (13%) cases and del17p13-in 8 (6%) cases. Complex karyotype was obtained in 8 (6%) patients. B-SLL was diagnosed in 11 (8%) patients; in 8 (73%) of them we identified del11q23, in 4 (36%)-trisomy 12. We did not reveal chromosome abnormalities in one of these patients. The combinations of del11q23 and trisomy 12 were found in 2 B-SLL patients. The frequency of del11q23 and trisomy 12 in B-SLL patients is higher than in B-CLL patients (p<0.0001 and p=0.02, respectively). There was no one B-SLL patient with del13q14, though it is the most frequent aberration in B-CLL patients. The amount of $CD38^+$ tumor cells 30% or more was typical for the majority of B-CLL patients with del11q23 (p=0.02), complex karyotype (p=0.031) and was also identified in all B-SLL patients. The amount of $CD38^+$ tumor cells less then 30% was characteristic for patients with del13q14 as a single aberration (p=0.045). 3% or more IgVH mutation was typical (p=0.039) for B-CLL patients without revealed aberrations. The IgVH data were obtained in 4 of B-SLL patients; all of them had less than 3% IgVH mutation. According to the survival rate of B-CLL/B-SLL patients with different karyotype we have allocated 3 prognostic groups of cytogenetic signs: favorable 'the absence of aberrations or del13q14 as a single abnormality; the group of intermediate prognosis-patients with del11q23 or trisomy 12; unfavorable-del17p13 or complex karyotype. The overall survival in all 3 patients groups differed each from other, p<0.025 (*see figure*). Conclusions. For B-CLL normal karyotype and del13q14 as a single aberration are the factors of favourable prognosis, del11q23 and trisomy 12 are the signs of intermediate prognosis, the unfavourable prognostic factors are del17p13 and complex karyotype. Cytogenetic features of B-SLL are del11q23, to a lesser degree - trisomy 12 and the absence of del13q14.



0794

IGVH MUTATIONAL STATUS, VH GENE USAGE, GENDER AND PROGNOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA

H.D. Alexander, C. Matthews, M.A. Catherwood, T.C.M. Morris,

Belfast City Hospital Trust, Belfast, Northern Ireland

Backgrounds. Somatic hypermutation in the variable region of the immunoglobulin heavy chain gene (IgVH) has been shown to be a powerful prognostic parameter in chronic lymphocytic leukaemia (CLL) and has the capacity to differentiate between two disease subsets. In recent years individual IgVH gene rearrangements, in particular VH3-21, have been shown to define unique disease entities in CLL. Aims. To determine the most commonly expressed VH genes in a Northern Irish cohort of CLL patients and to ascertain if variations in gender, light chain restriction and the presence of chromosomal abnormalities show associations with IgVH mutational subgroups and individual gene rearrangements. *Methods.* Two hundred and twenty-eight CLL patients were recruited from Belfast City Hospital and surrounding hospitals. IgVH mutational status and VH gene usage were determined using standard BIOMED-2 primers and protocol followed by sequence analysis. FISH analysis was performed to identify the presence of recurrent chromosomal aberrations. Light chain restriction was determined by standard immunophenotyping techniques. *Results.* The most common VH gene rearrangements were VH4-34 (13.5%), VH1-69 (12.3%), VH1-2 (7.9%), VH3-21 (7.9%) and VH1-3 (7.5%). Females showed a bias towards mutated IgVH status (2M: 1UM), overuse of VH4 genes (40%) and a lower frequency of trisomy 12 (7%). In contrast males showed no mutational bias (1:1), overrepresentation of VH1 genes (38%) and a higher incidence of trisomy 12 (21%). VH3-21, VH3-48 and VH3-53 showed preferential lambda light chain restriction, while VH1-69 had overrepresentation of kappa light chains. Further analysis of VH3-48 and VH3-53 gene usage showed a preponderance of females (7:1, 3:1 respectively), lambda light chain restriction and inferior outcome irrespective of IgVH mutational status. *Conclusions.* This study has demonstrated gender related differences in CLL, which can be explained partly by the increased incidence of mutated IgVH genes, biased use of VH4 genes and lower frequency of adverse chromosomal aberrations in female patients. This study confirms that gender related survival differences exist in CLL patients and that gender should be taken into account in risk stratification of patients at presentation. Furthermore, specific VH gene usage is associated with have distinctive characteristics, supporting the concept that antigens have an important role to play in the aetiology of CLL.

0795

VH GENE USAGE AND SOME FEATURES OF DISEASE COURSE IN CHRONIC LYMPHOCYTIC LEUKEMIA

N. Bilous,[1] I. Abramenko,[1] I. Kryachok,[1] Z. Martyna,[1] A. Chumak[1], I. Phylonenko,[2] T. Serbynenko[2]

[1]*Research Centre for Radiation Medicine, Kiev, Ukraine;* [2]*Regional Hospital, Poltava, Ukraine*

Backgrounds. Currently immunoglobulin heavy chain variable region (VH) gene mutation status considered to be the most accurate discriminator of clinical outcome in CLL. However, the latest data suggest, that individual VH genes might also have prognostic significance, independently of mutation status. *Aim*: The aim was to refine the significance of VH mutation status and VH gene usage for prognosis of CLL course. Methods. Total RNA was extracted by guanidinium thiocianate method from peripheral blood lymphocytes and reverse transcribed into cDNA. VDJ sequences were amplified using VH-FR1/JH primers as recommended by BIOMED-2 protocol. Purified PCR products were sequenced directly with automated DNA sequencer and sequences were analyzed using international databases (IMGT, IgBlast, V-BASE). *Results.* We sequenced VDJ rearrangements in 76 CLL patients from different regions of Ukraine. Of the 51 functional VH genes, 24 were identified, which represented 3 VH gene families -VH3 (34; 44.7%), VH1 (28; 36.8%), and VH4 (14; 18.4%). The most frequent VH gene was VH1-69 (19 cases, 25%) followed by VH4-34 and VH3-33 (5 cases each; 6.6%), VH3-07, VH3-30, VH3-09 and VH3-21 were used in 4 cases each (5.3%). Unmutated were 53 cases (69.7%) and 23 cases (30.3%) were considered as mutated ones using 2% mutation border. All VH1-69 cases had a germline configuration, and this gene was the most frequently represented VH gene in the unmutated subset. Within the mutated subset the most frequent gene was VH3-07. In comparison with mutated cases, patients with unmutated VH status had shorter median time to progression (40 months vs. 91 months; Log Rank 7.24; p=0.0071), more frequent marked lymphadenopathy and splenomegaly at the moment of diagnosis (13/53 vs. 0/23; p=0.008), though differences in total survival were non-significant (median 49 months for mutated cases and did not reach for unmutated cases; Log Rank 2.53; p=0.1115) due to a short time of observation. All patients resistant to therapy with progressive disease according to NCI-WG belonged to unmutated cases from VH1 (6) and VH3 family (6) (p=0.02). Autoimmune disorders were registered mainly among unmutated VH1 (3-VH1-69, 2-VH1-58, and 1-VH1-45) and VH3 cases (1- VH3-09, 1-VH3-11, and 1-VH3-21) and only one patient had mutated VH1-02 gene. Secondary cancer has developed in 2 patients with unmutated VH1-69 (stomach, urethra) and in one patient with mutated VH3-07 (skin). On the contrary, Richter transformation was observed only in patients with VH3 and VH4 genes (VH3-30 and VH3-48 mutated; VH3-09, VH3-20, VH3-33, VH3-53, and VH4-34 unmutated) (p<0.05 in comparison with VH1 gene family). The worth course of disease had 2 unmutated VH3-09 patients (dead, survival 11 and 49 months), and the best-the patient with mutated VH3-21 gene with short CDR3 sequence - ARDMNAMDV (alive, survival 247 months, in status of complete remission after fludarabine treatment, duration 26 months). *Conclusion*: While the significance of VH mutation status for prognosis of CLL course is beyond question, some features of the disease might be associated with individual VH gene usage.

0796

EFFICACY OF RITUXIMAB IN HAIRY CELL LEUKEMIA

M.K. Angelopoulou,[1] S.I. Kokoris,[1] P. Tsaftaridis,[1] E. Plata,[1] F.N. Kontopidou,[1] E.M. Dimitriadou,[1] T.P. Vassilakopoulos,[1] M.P. Siakantaris,[1] Z. Galani,[1] C. Kalpadakis,[1] S. Sachanas,[1] M.C. Kyrtsonis,[1] P. Korkolopoulou,[1] P. Panayiotidis,[1] G.A. Pangalis,[1] M.N. Dimopoulou[2]

[1]National and Kapodistrian University of, ATHENS, Greece; [2]University of Athens, ATHENS, Greece

Backgrounds. Hairy cell leukemia (HCL) is a rare B-chronic lymphoproliferative disorder with an indolent course. First-line treatment modalities include 2-chlorodeoxyadenosine, 2-deoxycoformycin and interferon-α. The efficacy of anti-CD20 (Rituximab) in other BCLDs and the strong expression of CD20 by hairy cell leukemia lymphocytes indicate that Rituximab could be an alternative in the treatment of HCL. *Aims.* The experience of a single Hematology Unit in the treatment of relapsed HCL with Rituximab. PATIENTS AND Methods. We retrospectively analyzed all hairy cell leukemia patients who received Rituximab as salvage therapy in 1st or subsequent relapse. The clinical and laboratory characteristics at diagnosis and relapse, primary treatment and its duration, as well as response to treatment were analyzed. Results. 10 patients treated with Rituximab were located among 110 patients diagnosed with HCL between 1980 and 2005. 9 were males and their median age at diagnosis was 46 years (range: 42-84). All but two patients presented with splenomegaly with a median spleen size of 8.5cm below left costal margin (range:2-30cm). 9/10 patients were leukopenic at diagnosis with the remaining one showing lymphocytosis. All patients displayed a typical immunophenotype from blood and/or bone marrow (CD20 strongly$^+$, CD19$^+$, CD22$^+$, FMC-7$^+$, CD11c$^+$, CD25$^+$, CD103$^+$). Four of them were CD23$^+$ and two CD10$^+$. Six patients received Rituximab at 1st relapse. Among them, one had received 2-deoxycoformycin as 1st line treatment, one 2-chlorodeoxyadenosine and four interferon-α as induction and maintenance. 3 patients had received more than one prior treatments. Two of them were IFN-α resistant and one had discontinued IFN-α due to side-effects. One patient received Rituximab at diagnosis, due to older age and possible complications with other therapies. The median time from diagnosis to Rituximab initiation for the 9 patients was 61 months (range: 19-168). Rituximab was administered at 375mg/m2 weekly for 6 cycles. Overall response rate was 77%. 2 patients went into complete remission, including a negative bone marrow biopsy, a negative immunophenotype and a negative immunoglobulin gene rearrangement. 2 patients achieved a complete hematologic remission with normalization of their cytopenias, but with remaining bone marrow infiltration of <25%. 3 patients achieved a partial hematologic response, while 1 patient was Rituximab resistant. One patient is not evaluable and the remaining one discontinued treatment after the first cycle due to the development of thrombocytopenia that was attributed to the drug. No other complications were recorded, except of mild infusion-related symptoms. Among the complete hematologic responders, no patient has relapsed with a median follow-up of 12 months (range: 4-38). Among partial responders, one achieved a complete response including a negative bone marrow and immunophenotype after Rituximab retreatment, one is alive in partial remission without the need of further therapy and one progressed within 5 months from Rituximab administration. Conclusions. Rituximab is a highly effective treatment for HCL in relapse with a response rate of 77%. Retreatment with Rituximab, or maintenance may be important, since ongoing responses are seen.

Quality of life and cost effectiveness

0797

SUCCESSFUL AND COST-EFFECTIVE PROPHYLAXIS AND TREATMENT OF TUMOR LYSIS SYNDROME (TLS) WITH LOW DOSES OF RASBURICASE

M. Hummel, D. Buchheidt, A. Adam, M. Kripp, L. Sadikaj, S. Reiter, R. Hehlmann

III. Medizinische Klinik Mannheim, Mannhein, Germany

Backgrounds. TLS commonly occurs in patients with hematologic malignancies and is characterized by elevation of uric acid, potassium and phosphate and by hypocalcemia. A major complication is renal failure caused by precipitation of uric acid and / or calcium phosphate crystals. Treatment consists of hydration, correction of electrolyte disturbances and lowering of uric acid. Rasburicase, a recombinant urate oxidase, has proven to be highly effective in lowering serum uric acid levels, and its application is not restricted in patients with renal failure. Costs for a full seven-day course of rasburicase in the dosage recommended are high and amount to approximately 4800 . For rasburicase a 86% reduction of uric acid levels 4 hours after the first dose has been reported. Therefore, the question arose whether lower doses of rasburicase than those recommended by the manufacturer can be applied for efficient prophylaxis and treatment of TLS. Patients and Methods. 40 patients (27 male, median age 65 yrs, range 16-88) received rasburicase in low doses for prophylaxis or treatment of TLS. Ten patients had acute leukemia, 6 had myeloproliferative disorders, 5 patients had high-grade Non-Hodgkin's lymphoma, 15 had low-grade Non-Hodgkin's lymphoma and 4 patients had solid tumors. TLS was classified into laboratory and clinical TLS and graded according to Cairo and Bishop. Thirty-five patients had elevated serum creatinine levels, the median creatinine level in these patients was 2.63 mg/dL (1,18-8.61 mg/dL). Laboratory TLS was diagnosed in 5 patients, 28 patients had clinical TLS. *Results.* Seven patients received rasburicase for prophylaxis of TLS. The mean LDH level in this group was 706 U/l, the mean uric acid level was 11.7 mg/dl before application of rasburicase. The median number of doses of rasburicase applied was 2 (range 1-2), the median total dose was 3 mg (0.052 mg/kg). After application of rasburicase the mean uric acid level decreased by 66% and was 3.7 mg/dl. None of the patients developed TLS. Thirty-three patients received rasburicase for treatment of TLS. The mean LDH level was 1668 U/l. The mean uric acid level was 15.8 mg/dl before application of rasburicase. The median number of doses of rasburicase applied was 1 (range 1-5), the median total dose was 3 mg (0.038 mg/kg). After application of rasburicase the mean uric acid level decreased by 80% and was 3.2 mg/dl. No patient required renal replacement therapy. Rasburicase was well tolerated by all patients without side effects. *Conclusion*. We applied rasburicase doses as low as 3,2-4,3% of the dose recommended by the manufacturer. Rasburicase applied in low doses proved to be effective for prophylaxis and treatment of TLS, even in patients with renal failure. For some patients doses as low as one vial of 1,5 mg of rasburicase was sufficient to control hyperuricemia, lowering the costs to 83. Cost-effective treatment becomes an increasingly important issue regarding limited budgets in health care. Further studies have to be conducted to establish dosing regimens for different clinical settings.

0798

COST-EFFECTIVENESS OF LENALIDOMIDE IN THE TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA (MM) PATIENTS IN THE UNITED KINGDOM

C. Schaefer,[1] F. Goss,[1] A. Szende,[1] G. Martinelli,[2] G. Morgan[3]

[1]*Covance Market Access Services, Gaithersburg, USA;* [2]*Institute of Hematology, Bologna, Italy;* [3]*Royal Marsden Hospital, Sutton, United Kingdom*

Backgrounds. Despite moderate therapeutic advances in recent years, improving progression-free and overall survival remains a key challenge in the treatment of MM. Revlimid™ (lenalidomide) is a new immunomodulatory drug, which is supplied in named patient programs (NPP) in relapsed or refractory MM patients in Europe, including the UK. We conducted a preliminary assessment of the cost-effectiveness of lenalidomide from the perspective of the UK's National Health Service (NHS). *Methods.* A comprehensive health economic decision analytic model was developed to evaluate and compare the costs and health benefits of lenalidomide plus dexamethasone versus bortezomib. The model combined data from three large-scale randomized clinical trials: two studies compared lenalidomide plus high-dose dexamethasone versus high-dose dexamethasone alone (MM-009 and MM-010 studies, total of n=691 patients), and one study compared bortezomib with high-dose dexamethasone alone (APEX study, n=669 patients). The model also built on published literature on additional parameter estimates and was validated by two UK hematologists. The model incorporated direct medical costs related to drugs, drug administration, diagnostic tests, office visits and other medical resource use associated with standard care and complications due to disease or adverse events. Unit cost estimates were based on NHS Trust/Primary Care Trust (PCT) National Reference Costs. Health outcome measures included time to progression and estimated overall survival. The time horizon of the analysis was time from treatment initiation until death. The main cost-effectiveness outcome measure used was cost per life year gained. *Results.* Baseline patient characteristics were comparable across the three trials. The weighted average of the proportion of patients with 2 or 3 prior treatments at baseline was 59.7%, 60.0%, and 61.9%, in the lenalidomide/dexamethasone, bortezomib, and dexamethasone arms, respectively. The median time to progression was longest with lenalidomide/dexamethasone (13.6 months), followed by bortezomib (6.2 months), and was shortest with the dexamethasone alone (4.3 months) strategy. As overall survival data for the lenalidomide/dexamethasone group was not yet available, the model conservatively assumed a 19.2-month additional survival, similar to what was observed for bortezomib patients after progression. Cost estimates assumed best supportive care treatment during extended survival. In base-case analysis that assumed NPP price of lenalidomide, the total cost of therapy until death was higher with lenalidomide/dexamethasone than bortezomib due to longer treatment duration (£56,942 vs. £34,795). The incremental cost-effectiveness ratio of lenalidomide/dexamethasone versus bortezomib was £35,915 per life year gained. The cost-effectiveness of lenalidomide/dexamethasone versus dexamethasone and bortezomib versus dexamethasone were similar. Based on a range of assumptions on survival with dexamethasone after progression if patients are not crossed over to lenalidomide or bortezomib (2.5 to 15 months), the cost-effectiveness ratio ranged between £ 22,351 - £38,451 and £16,955- £41,529 per life year gained, respectively. Conclusions. These results suggest that lenalidomide can be viewed as a cost-effective treatment option in the UK when considering the orphan disease status of relapsed or refractory MM. Further research is warranted to confirm these findings, as sufficient follow-up data from the lenalidomide trials will be reached to estimate overall survival time in responding MM patients.

0799

IMPROVEMENT IN HEMOGLOBIN LEVELS AND QUALITY OF LIFE IN ANEMIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES RECEIVING EPOETIN α DURING CHEMOTHERAPY: RESULTS FROM THE EPOLYM TRIAL

C. Gisselbrecht,[1] N. Marschner,[2] G. Avvisati[3] for EPOLYM Investigators[4]

[1]*Hopital Saint Louis, Paris, France;* [2]*Outpatient Cancer Center, Freiburg, Germany;* [3]*Universit Campus Biomedico, Rome, Italy;* [4]*University Hospitals and Clinics, Europe, Switzerland*

Backgrounds. Anemia from disease and/or chemotherapy is a common complication in patients with Hodgkin's disease (HD), non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM). Because anemia is associated with fatigue and other symptoms of diminished quality of life (QOL), treatment of anemia in patients with hematologic malignancies is important. *Aims.* The primary objective was to compare change in QOL from baseline to week 12 when anemia is corrected with a once-weekly (QW) regimen of epoetin alfa in patients with HD, NHL, CLL, or MM receiving chemotherapy. Improvements in Hb levels and transfusion requirements were also recorded. *Methods.* EPOLYM was an international, multicenter, open-label, Phase IIIb, 24-week trial in anemic (hemoglobin [Hb] <=12 g/dL) cancer patients receiving chemotherapy. Patients received epoetin alfa 40,000 IU QW subcutaneously to a target Hb of 11.5-13.0 g/dL with dosage adjustments based on clinical response. The primary objective of QOL improvement was evaluated by the Functional Assessment of Cancer Therapy-Anemia (FACT-An) which measured functional well-being (FWB), fatigue subscale score (FATS), non-fatigue subscale score (NFATS), and total anemia subscale score (ANS). The Linear Analog Scale Assessment (LASA; also known as the Cancer Linear Analog Scale [CLAS]) for energy level, daily activities, and overall QOL was also employed. Statistical significance was analyzed by the paired sample t-test. Changes in Hb level and transfusion requirements from baseline were evaluated. *Results.* Intent-to-treat population was 1034 patients: 416 NHL, 307 MM, 165 HD, and 145 CLL (1 unspecified). Epoetin alfa dosage was increased for 37.8% of patients. Mean baseline FACT-An scores were: FWB 14.9; FATS 31.3; NFATS 19.4; ANS 50.7. Mean increases for FACT-An from baseline to week 12 and baseline to week 24 were significant for all parameters: FWB (p=0.005); FATS (p<0.001); NFATS (p<0.001); ANS (p<0.001). Mean baseline LASA scores ranged from 46.3'50.4 mm (normal: 70'100 mm) indicating poor QOL at study initiation. Mean changes in LASA at week 12 were clinically* and statistically significant: energy level, 8.9 mm (p<0.001); daily activities, 7.5 mm (p<0.001); overall QOL, 6.8 mm (p<0.001); improvements were even greater at 24 weeks for all parameters. Both FACT-An and LASA scores improved as Hb level increased, with increases >2 g/dl demonstrating the greatest mean improvements at week 12 and 24. Baseline mean Hb for combined diagnostic groups was 10.4 g/dL; patients with MM had the lowest mean Hb (10.0 g/dL) and patients with HD had the highest mean Hb (10.8 g/dl). Mean Hb increases after 3-5, 12, and 24 weeks of epoetin alfa treatment were 1.0 g/dL, 1.7 g/dL, and 1.7 g/dL, respectively (p< 0.001 at all time points). MM patients had the greatest Hb increase at week 12 (2.1 g/dL). Transfusions were administered to 25% of patients during the study. *Conclusions.* Treatment with epoetin alfa resulted in improved QOL in anemic patients with HD, NHL, CLL, and MM. This improvement was associated with increases in Hb attained with epoetin alfa 40,000 IU QW.

**Patrick DL, et al. Eur J Cancer 2003;39:335-45*

0800

COST AND COST-EFFECTIVENESS OF FLUCAM VERSUS FCR IN PATIENTS WITH RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

S.T Thompson,[1] F.N. van Nooten,[2] M.A. van Agthoven,[3] P.H. Huijgens[4]

[1]*Schering AG, Berlin, Germany;* [2]*MC-University Medical Centre, Rotterdam, Netherlands;* [3]*Erasmus MC-University Medical Centre, Rotterdam, Netherlands;*[4]*VU University Medical Centre, Amsterdam, Netherlands*

Backgrounds. The most promising approaches for treatment of relapsed CLL involve the use of drug combinations with complimentary mechanisms of action against the disease, and immunochemotherapy combinations have proven to be particularly effective. However, these combination therapies may also be associated with higher costs, and in any treatment decision cost needs to be weighed against the added therapeutic benefit. *Aim*. This analysis estimates the likely cost and compares cost-effectiveness of the emerging fludarabine + alemtuzumab (FluCam) combination versus the fludarabine, cyclophosphamide, and rituximab (FCR) regimen in relapsed/refractory CLL patients. *Methods.* The effectiveness evidence used for analysis was derived from 2 published phase II clinical studies evaluating FluCam or FCR in the relapsed CLL setting: Elter *et al.* 2005 and Wierda *et al*. 2005. Overall response rates (ORR) and *expected months in remission per patient treated* were outcome measures used to assess the effectiveness or *health benefit* of each drug combination; the latter was estimated by multiplying a weighted ORR by the duration of response. Resource use associated with each intervention was based on published literature, a previous patient level costing study conducted in the Netherlands in follicular lymphoma, and expert opinion. Unit costs for the Netherlands were derived from local hospital accounting systems, published tariffs, and wholesale prices listed for 2003, the price year chosen for this analysis. Sensitivity analyses were

conducted to test the robustness of the assumptions. *Results.* The mean total cost of FluCam and FCR treatment was estimated at approximately 26,426 and 35,324, r espectively, assuming 6 cycles of therapy. The ORR reported for FluCam is 83% and for FCR it is 73%, which would yield a cost per responder of 31,838 and 48,389, r espectively. Data on response duration for either therapy are currently not available, but preliminary estimates of time to progression (TTP) are 13 months for all patients and 28 months for responders. Assuming as a base-case scenario a 73% ORR and 24 months of response duration for each therapy, then the expected number of months in remission per patient treated would be 17.52 months, or 1.46 years. Comparing costs and assuming a similar level of clinical benefit, the cost per year in remission for FluCam and for FCR would be 18,100 and 24,194, r espectively. Sensitivity analyses suggest that FCR would need to be at least 25% more effective than FluCam to achieve equivalence in terms of cost-effectiveness, which is unlikely given that alemtuzumab is more effective than rituximab as a monotherapy. *Conclusions.* Based on this analysis, FluCam is potentially a more cost-effective treatment strategy for relapsed/refractory CLL. The findings of this analysis imply that for each relapsed CLL treatment where FluCam is used rather than FCR, the cost savings to the payer would be on average ≥8,898 if both therapies were equivalent in terms of efficacy, and even more if FluCam is the more effective alternative. Randomized trials comparing the effectiveness and cost of both combinations are needed to confirm the findings of this analysis.

0801

DYSMETABOLIC SYNDROME IN YOUNG SURVIVORS OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDHOOD

M. Moschovi,[1] G. Trimis,[1] G. Chrousos,[2] I. Papassotiriou,[3] F. Tzortzatou-Stathopoulou[1]

[1]*Athens University, Athens, Greece;* [2]*First Pediatric Dep, Athens University, Athens, Greece;* [3]*'Aghia Sophia' Hosp, Athens, Greece*

Backgrounds. An increased incidence of obesity and cardiovascular morbidity and mortality was recently observed in adult survivors of childhood malignancies younger than 45 years. Aim: To investigate the presence of the dysmetabolic syndrome in a population of young survivors of acute lymphoblastic leukemia (ALL) in childhood. *Methods.* We enrolled randomly 80 patients with ALL (50 males) diagnosed between 1991 and 2002. The median age at diagnosis was 5.2 years (range, 1-14.3 years), at time of study entry 13.9 years (range, 5.2-24.1 years), while the median interval since completion of chemotherapy was 6.3 years (range, 1.1-12.2 years). Sixty-two patients (Group A) received only chemotherapy, while 18 patients (Group B) received chemotherapy and cranial irradiation (18 Gy). We included epidemiological, clinical and laboratory indices. Results. Diet excessive in calories, lipids and refined carbohydrates was reported by 59/80 patients (74%), while absence of regular physical activity by 51/80 (64%). Frank obese (BMI>30) were 20/80 patients (25%), overweight (BMI>25) were 35/80 (44%), increased systolic and/or diastolic BP (>90th percentile) was detected in 17/80 (21%), increased serum TG (>95th percentile) in 17/80 (21%) and reduced serum HDL-C concentrations (<5th percentile) in 10/80 (12%). Slightly elevated FFA were detected in 15/80 patients (19%) and hepatic enzymes in 9/80 (11%). Increased fasting insulin was seen in 6/80 (8%) and HbA1c in 12/80 (15%), however, fasting blood glucose was normal in all the patients. Osteopenia was detected in 57/80 patients (71%) and increased C-reactive protein (CRP) and/or serum amyloid A (SAA) in 13/80 (16%). Finally, reduced IGF-1 was detected in 12/80 patients (15%) and thyroid hormone abnormalities in 9/80 (11%), only in Group B. There is a statistical significant increase in the prevalence of obesity (p=0.024), hyperinsulinemia (p=0.004) and finally of dysmetabolic syndrome (p=0.017) in Group B. Furthermore, there is a statistical significant increase in the prevalence of obesity (p=0.017), high serum triglycerides (p=0.023), low serum HDL-C (p=0.032), hypertension (p=0.022) and dysmetabolic syndrome (p=0.028) for the total group. Overall, 9/80 subjects (11%) have already manifested dysmetabolic syndrome, 8% of Group A (5/62) and 22% of Group B (4/18). The mean value of BMI, fasting insulin and TSH was increased in Group B, while the mean value of IGF-1 was decreased. BMI is positively correlated with systolic BP (0.626, p=0.01), fasting insulin (0.454, p=0.018), serum triglycerides (0.516, p=0.015) and inversely correlated with _DL-C (-0.546, p=0.014) and IGF-1 (-0.719, p=0.003). Fasting insulin is positively correlated with serum triglycerides (0.602, p=0.01) and inversely correlated with HDL-C (-0.473, p=0.017) and IGF-1 (-0.696, p=0.005). Finally, CRP is positively correlated with BMI (0.266, p=0.037). *Conclusions.* Young survivors of childhood ALL, especially those treated with cranial irradiation, are at risk for obesity, dyslipidemia, insulin resistance, hypertension and, finally, dysmetabolic syndrome.

0802

PAIN IN HAEMATOLOGY: AN OUTCOME RESEARCH PROJECT TO EVALUATE THE EPIDEMIOLOGY, PATHOPHYSIOLOGY AND EFFECTS OF PAIN TREATMENT IN IN-HOSPITAL PATIENTS

P.N. Niscola,[1] C.L. Cupelli,[2] R.C. Romani,[3] G.M. Giovannini,[4] P.D. Piccioni,[1] O.L. Ottaviani,[2] M.M. Mirabile,[2] T.A. Tendas,[2] N.B. Neri,[2] T. Dentamaro,[1] M.L. Maurillo,[1] R. Marra,[4] E. Angelucci,[3] P. De Fabritiis[2]

[1]*Sant'Eugenio Hospital, ROMA, Italy;* [2]*Tor Vergata University, Rome, Italy;* [3]*Businco Oncological Hospital, Cagliari, Italy;* [4]*Frosinone Hospital, Frosinone, Italy*

Background. Management of pain associated with blood-related diseases is often difficult and inadequate, notwithstanding the availability of several therapeutic interventions and of well-known guidelines and protocols. *Patients and Methods*. A six months multicenter study involving the wards of three Italian haematological Centres was started in October 2005 to investigate the epidemiology and the clinical characterisation of pain in haematological patients. A treatment protocol based on the cancer pain WHO analgesic ladder was applied. Data reported as January 2006 included 286 patients (166 male) with a median age of 67 (19-89) years. The total number of admissions during the study period was 415. Haematological diagnoses were as following: 85 (30%) non-Hodgkin's lymphomas, 55 (20%) acute myeloblastic leukaemia (AML), 29 (10%) multiple myeloma (MM), 16 (5%) acute lymphoblastic leukaemia (ALL), 29 (10%) other lymphoproliferative disorders, 26 (9%) myelodisplastic and chronic myeloproliferative disorders and 46 (16%) non-malignant diseases. *Results*. Of 286 patients, 128 (45%) experienced almost one pain syndromes, for a total of 174, which pathophisiology were diagnosed as follows: 76 (43,6%) deep somatic, deriving from the bone in most cases, 34 (19,5%) superficial somatic (mucositis and cutis derangements), 30 (17,2%) visceral, 15 (8,6%) neuropathic, 15 (8,6%) mixed or by unknown mechanisms and 4 (2,2%) iatrogenic or infection related. A diagnosis of MM, ALL and AML, and an advanced disease phase were significantly associated with a higher incidence of pain. The treatment protocol was based on three steps, according to the intensity of pain. The first step (mild pain) included paracetamol, the second step (moderate pain) tramadol, and the third step (severe pain) morphine. In selected cases, oxycodone or fentanil patches were used in the place of morphine to treat severe pain. Of 174 pain syndromes, 48 (28%), 49 (29%) and 77 (53%) pain syndromes were treated with a first-line therapy according to the first, the second and the third step respectively. Of the 97 pain syndromes treated according the first two steps, 37 (38%) needed a treatment escalation to the third step after 4 (1-12) days. No serious adverse effects were recorded. The adopted treatment approach, integrating causal interventions (if applicable) and analgesic measures, including the adjuvants to treat neuropathic pain, allowed a prompt relief in more than 90% of the pain syndromes. No serious adverse effects were recorded. *Conclusion*. These preliminary results indicate that, in the setting of haematological wards, pain is a significant symptom requiring prompt medical attention. Moreover, our data outline that most pain syndromes can be effectively controlled by the currently available treatment strategies. Therefore, the implementation of clinical pathways and standardized protocols based on well-defined algorithms can provide the auspicial advancements toward a 'pain-free' haematological hospital.

0803

COST-EFFECTIVENESS OF ^{90}Y-IBRITUMOMAB TIUXETAN (^{90}Y-ZEVALIN) VERSUS RITUXIMAB MONOTHERAPY IN PATIENTS WITH RELAPSED FOLLICULAR LYMPHOMA

S.T. Thompson,[1] F.N. van Nooten,[2] M.A. van Agthoven[3]

[1]*Schering AG, Berlin, Germany;* [2]*MC - University Medical Centre, Rotterdam, Netherlands;* [3]*Erasmus MC University Medical Centre, Rotterdam, Netherlands*

Backgrounds. Currently, there are limited cost-effectiveness data comparing the use of 90Y-Zevalin with rituximab in follicular lymphoma (FL). Aims. The objective of this analysis was to estimate the (incremental) cost-effectiveness of a single dose of 90Y-Zevalin 0.4 mCi/kg compared with: 1) standard rituximab treatment of 375 mg/m^2 weekly for 4 weeks (4-dose rituximab); and 2) standard rituximab followed by 4 weeks of maintenance therapy (8-dose rituximab) in patients with FL. *Methods.* The effectiveness data used for the analysis were derived from the only 2 clinical studies published to date enrolling comparable populations where patients had received either ^{90}Y-Zevalin or rituximab monotherapy. These were a randomized trial of ^{90}Y-Zevalin versus 4-dose rituximab,

the results of which were published by *Gordon et al. (2005)*, and, for the 8-dose rituximab arm, a study published by *Ghielmini et al. (2004)*. Expected months in remission were used as the measure of effectiveness for the analysis. Months in remission were estimated by multiplying the overall response rates for each therapy by the response duration. To estimate the resources involved in the management of adverse events, we consulted a panel of experts. All other resource use estimates, ie, those for administration, prophylaxis, and monitoring, were derived from clinical guidelines and the randomized trial database. Unit costs for Netherlands were derived from local hospital accounting systems, tariffs, and listed wholesale prices for medication. The price year was 2003 for all costs except 90Y-Zevalin, for which it was 2006. *Results.* The mean total cost of treatment with 90Y-Zevalin was estimated to be approximately EUR 18,274. The mean total cost of treatment with 4 doses of rituximab was estimated to be lower at EUR 9,847, whereas the cost associated with an 8-dose rituximab treatment was EUR 20,112. In terms of health benefits, the average number of disease-free months per patient treated was highest for 90Y-Zevalin at 14.4 months followed by 11.4 months for the 8-dose rituximab and 6.2 months for the 4-dose rituximab. When the estimates of health benefit are combined with costs, the analysis demonstrates a mean cost per disease-free month for 90Y-Zevalin of EUR 1,272, the lowest of the 3 therapies, followed by EUR 1,599 for 4-dose rituximab therapy, and EUR 1,770 for 8-dose rituximab. *Conclusions.* The findings imply that for each third-line follicular NHL treatment where 90Y-Zevalin is used rather than 4-dose rituximab, the additional cost to the payer would be, on average, EUR 8,426. For this additional cost, the benefit to the patient would be an average 8.2 additional months in remission, over and above what would have been gained with 4-dose rituximab therapy. Furthermore, when the costs and benefits of ^{90}Y-Zevalin are compared with the 8-dose rituximab regimen, ^{90}Y-Zevalin is the more cost-effective strategy.

0804

COST-EFFECTIVENESS OF ONCE-DAILY ORAL CHELATION THERAPY WITH DEFERASIROX VERSUS INFUSIONAL DEFEROXAMINE IN TRANSFUSION-DEPENDENT THALASSEMIC PATIENTS: A BRAZILIAN PERSPECTIVE

A. Calabro,[1] T.E. Delea,[2] O. Sofrygin,[2] T.D. Coates,[3] P. Phatak,[4] A. Arajo[5]

[1]*Novartis Biociéncias S.A., Sao Paulo, Brazil;* [2]*Policy Analysis Inc. (PAI), Brookline, Massachusetts, USA;* [3]*Childrens Hospital of Los Angeles, Los Angeles, CA, USA;* [4]*Rochester General Hospital, Rochester, NY, USA;* [5]*Hemope, Recife, Brazil*

Background. Transfusion-dependent thalassemic patients require chelation therapy to treat iron overload and prevent its complications including cardiac, endocrine and hepatic toxicity. Deferoxamine (DFO) is an effective iron chelator, but must be administered as an 8-12 hour infusion 5-7 times per week, leading to poor compliance and/or reduced quality of life. Deferasirox (DSX) is a novel once-daily oral iron chelator with a high iron-binding potency and selectivity that has recently been approved by the FDA, Swissmedic and ANVISA in Brazil. Clinical studies with DSX have demonstrated efficacy for the treatment of transfusional iron overload. Cost-effectiveness analysis is a technique used to determine whether the benefits of new therapies are worth their additional costs. The objective of this analysis was to evaluate, from a Brazilian perspective, the cost-effectiveness (CE) of DSX vs DFO in patients with transfusion-dependent thalassemia. Methods. A decision-analysis model was used to estimate the total additional lifetime costs and quality-adjusted life year (QALY) gained with DSX versus DFO in patients with transfusion-dependent thalassemia (≥8 transfusions per year). Compliant patients were assumed to receive dosages of DSX and DFO that have been shown to be similarly effective in such patients. Probabilities of complications of iron overload and death by average compliance with chelation were estimated using data from published studies. Compliance with DFO was based on analyses of US health insurance claims data of transfusion-dependent thalassemic patients. Cost of cardiac disease was taken from a published local study in the public healthcare system. The costs of other complications of iron overload conservatively were not included in the model due to lack of local data. Because data on compliance with DSX in typical clinical practice are unavailable, we used published data on compliance with the three-times-daily oral chelator deferiprone vs DFO. Utilities (weights representing patient quality of life) were obtained from a study that used time-trade-off techniques to measure patient preferences for oral vs infusional chelation therapy, as well as published literature and assumption. The analyses were based on the anticipated cost of DSX and the current DFO cost for public payers excluding taxes. The administration cost of DFO was calculated from the patient perspective using the Brasíndice Table for syringes, needles, scalp and other materials. For the standard infusion pump (Infusa T Medis) was used the price to consumer from a local distributor. Results were generated for DFO-naive patients (age 2 years, no prior DFO therapy). Costs and QALYs were discounted at 3% annually. Costs were reported as 2006 US dollars. *Results.* The cost of DFO administration was $195 per month representing 22% of the cost of chelation therapy with DFO. In DFO-naïve patients, DSX results in a gain of 3.8 QALYs per patient. These benefits are obtained at an additional expected lifetime cost of $90,515 per patient. CE is $23,425 per QALY gained. *Conclusion*. Assuming a cost-effectiveness threshold of less than three times the GDP per capita (WHO, 2002) and that the GDP per capita in Brazil is $8,500 (2005), deferasirox is a cost-effective use of the healthcare resources in patients with transfusion-dependent thalassemia.

0805

COST EFFECTIVENESS OF ADDING IMATINIB TO CHEMOTHERAPY IN ADULT PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA: AN EXPLORATORY ANALYSIS

W. Feng,[1] T. Tran,[2] M. Stephens,[2] F. Botteman[2]

[1]*Novartis Pharmaceuticals Corporation, Florhem Park, USA;* [2]*Pharmerit, Bethesda, USA*

Backgrounds. The prognosis of Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ALL) in adults is extremely poor. Imatinib has been reported to successfully induce and consolidate remissions and extend disease-free survival in Ph^+ ALL patients with good tolerability in clinical studies. *Aims.* This study explores the cost effectiveness of imatinib plus conventional chemotherapy regimens v. conventional chemotherapy alone in adult Ph^+ALL patients. Methods. A Markov model was used to follow a hypothetical cohort of 1000 adult Ph+ALL patients receiving imatinib plus conventional chemotherapy (CC) or CC alone. Patients were modeled for a total of ten years in monthly intervals. Patients were distributed over time into three health states: alive without disease progression (DFS), alive with disease progression (DS), or dead. For the purpose of this model, patients were assumed to continue imatinib therapy (600 mg daily) for up to two years as long as they remained in the DFS state. Probabilities of being in the states were derived from the published literature of case series for patients with Ph^+ALL receiving either treatment. In the absence of relevant data pertaining to Ph^+ALL, assumptions about costs and utilities were derived from a cost analysis of chronic myeloid leukemia. Only direct medical costs were included in the analysis using a U.S. health care payer perspective. All outcomes were discounted at a 3% rate per annum. *Results.* Based on the literature, the median disease free survival and overall survival of Ph^+ALL adult patients with CC were 6 and 9 months (*Thomas X et al. 1998*), respectively. The 12-month disease free survival and survival for imatinib+CC were 72% and 84% (Towatari 2004), respectively. Total discounted survival was 1.02 years for CC and 4.29 years for imatinib+CC. Total discounted disease free survival was 0.76 year for CC and 2.79 years for imatinib+CC. Assuming utility weights of 0.854 and 0.596 for DFS and DS, respectively, the total discounted quality adjusted life years (QALY) were estimated to be 0.85 v. 3.33 for CC and imatinib+CC, respectively. Thus, the net incremental gain in discounted quality adjusted survival was 2.47 QALYs. Total incremental treatment costs for imatinib+ CC were $102,507 as compared to CC over 10 years. Therefore, the incremental cost per QALY of imatinib+CC v. CC alone was approximately $41,500 (i.e., $102,507 divided by 2.47 QALYs) which is within the range of usual acceptable cost effectiveness threshold. *Conclusions.* For adult ALL patients with poor prognosis due to Ph+ALL, our exploratory analysis suggests that, given the underlying data and assumptions, adding imatinib to current chemotherapy regimens may be cost-effective compared to chemotherapy alone.

0806

MASS NEONATAL CORD BLOOD SCREENING: COST-EFFECTIVE IDENTIFICATION OF HEMOGLOBINOPATHIES

V. Pathare,[1] S. Al Kindi,[1] S. Al Zadjali,[1] Q. Al Abri,[1] H. Al Haddabi[1], S. Muralitharan,[1] M. Mathews,[1] D. Dennison,[1] R. Krishnamoorthy[2]

[1]*Sultan Qaboos University, MUSCAT, Oman;* [2]*Hopital Robert Debre, Paris, France*

Backgrounds. The diagnosis of haemoglobinopathies is of growing importance particularly in countries with high incidence of disorders of globin chain synthesis, both thalassemias and structurally abnormal hemoglobins. Sickle cell disease is a major public health problem in the Sultanate of Oman with a high rate of morbidity and mortality. The overall prevalence of Sickle cell trait of 6%[2.9-10%] whereas that of tha-

lassaemia trait is 2-3%. *Aims.* To ascertain the feasibility of neonatal cord blood screening in the Sultanate of Oman in an effort to determine the prevalence of haemoglobinopathies by a cost-effective method. *Methods.* A total of 1575 cord blood samples were screened for presence of possible haemoglobinopathies by high performance liquid chromatography[HPLC] technique using Biorad Variant™ program between April 2005 & February 2006. Complete blood counts were also obtained on Cell Dyn 4000 automated blood cell counter. All samples were then processed to isolate and store mononuclear leukocytes for subsequent molecular diagnostics. Results. The findings indicated a 37.21% incidence of α-thalassaemia [predominantly - α3.7/αα]. Furthermore, the overall incidence of other haemoglobinopathies was 10.38% with 6.45% and 2.36% incidence of sickle haemoglobin and β thalassaemia respectively. On HPLC, D-window, E-window and C-window were present in 1.11%, 0.33% and 0.13% of the samples respectively, with a few samples presenting with unknown peaks that need further studies. *Summary/Conclusions.* The wealth of information obtained by screening highlights the significantly high incidence of haemoglobinopathies in newborns in the Sultanate of Oman and emphasizes the value of neonatal cord blood screening to be implemented as the first step in the national strategy towards prevention of haemoglobinopathies. Although cord blood screening cannot give a definitive diagnosis it can identify the small group of neonates that require further testing. Moreover, the cost of testing per sample was approximately 1 Euro.

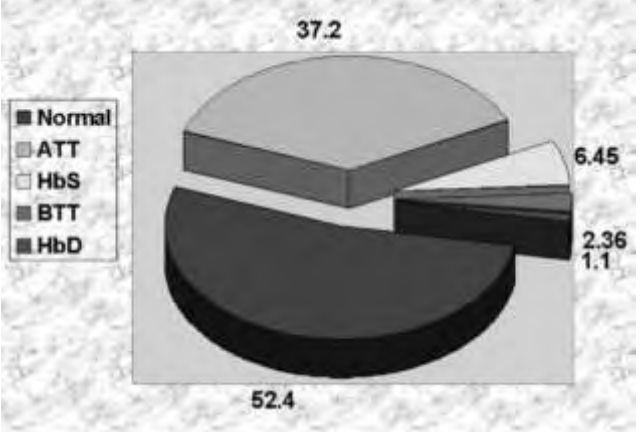


Figure 1. Prevalence of hemoglobinopathies in the newborn.

0807

FOLLOWING THE CASE OF ESSENTIAL THROMBOCITOSIS CAUSED BY NOURISHING DEFICIENCY

C. Malem

Laboratory Claudio Malem & Associated, GENERAL ALVEAR, Argentina

Thirty two male longevous patients, residents in an old people's home and abandoned by their relatives, were studied. At the start of this case-study the patients had their blood values, which showed severe anaemia and microcitosis accompanied with trombocitosis, badly altered. It was decided to move the old men to another home, where the nourishing diet was radically changed, leaving out the former one which consisted of carbohidrates the seven days of the week and changing it for the ingestion of protein, vegetables and diary products nutriments. During the first forty five days they were helped with ferrous anhidro succinate to raise the level of hemoglobin, whose value was between seven and eight g/dl (in average). At the next blood control it was clear-sighted that slowly and gradually the values were encouragingly changing, that's why it was decided to suspend the ferrous anhidrous succinate and allow the new diet to perform the corresponding supply. After 90 days, at the corresponding blood control, it was shown that the patients had got normal analytical values, recovering their health, vitality and basically their right to enjoy completely a better quality of life, thus achieving psicho-social stability.

0808

VALUE OF TRANSFUSION-FREE LIVING IN MDS: RESULTS OF HEALTH UTILITY INTERVIEWS WITH PATIENTS

A. Szende,[1] C. Schaefer,[1] F. Goss,[1] M. Wang,[2] K. Heptinstall[3]

[1]*Covance Market Access Services, LEEDS, United Kingdom;* [2]*Celgene Corporation, SUMMIT, USA;* [3]*MDS Foundation, CROSSWICKS, USA*

Backgrounds. Achieving transfusion independence in patients with transfusion-dependent MDS has been defined as a key treatment goal in clinical trials of new interventions and in everyday clinical practice as new treatments have become available. However, data is lacking on how patients themselves value health states associated with transfusion-independence as opposed to transfusion-dependence. *Methods.* We performed health utility interviews with MDS patients in the US, France, and the UK to elicit the value of transfusion-independence or reduced transfusion burden compared to transfusion dependence (i.e., three distinct health states). Health state descriptions were developed based on literature and reports from MDS patient focus group discussions, and were validated by a haematologist. Each health state card included different severity/intensity of problems on the following quality-of-life (QoL) domains: reliance on blood transfusions and health care provider facility; need to arrange one's life around medical appointments; fatigue and tiredness that limits performce of routine physical activities; interference of disease with social and family life; worry about the future due to health condition; discomfort associated with medical conditions and treatment, and the feeling of being at risk of infection; reliance on support persons for self care and routine activities; feelings of being a burden to family; and feeling sad, hopeless, and helpless. Face-to-face interviews used the Feeling Thermometer visual analogue scale (VAS) and the Time Trade-off (TTO) method to value the health states on a scale anchored on 1 (perfect health) and 0 (dead). We administered background questionnaires on socio-demographic, clinical, and QoL (EQ-5D) characteristics to describe the patient sample. *Results.* Thirty-eight MDS patients in the US (n=8), France (n=9), and UK (n= 21) completed the interview. The mean age was 66 years (range: 29-83); 55% were male. The majority were retired (66%), had secondary/high school education (38%) or higher (24%), and were living with family, a partner or spouse, or friends (76%). The mean time from MDS diagnosis was 5 years (range: 1-23). The majority of patients received blood transfusion(s) previously (87%), and 47% had received a blood transfusion in the last three months. Mean EQ-5D utility score was 0.78, and patients reported at least some problem with mobility (44%), usual activities (39%), pain/discomfort (47%), and anxiety/depression (29%). One patient reported problems with self-care. Few patients had difficulty understanding the rating scale (n=3) and TTO (n=3) exercise. The health utility score for the transfusion-independent health state was significantly higher than for health states with reduced transfusion requirement (0.85 vs. 0.77, $p<0.001$), and transfusion dependence (0.85 vs. 0.62; $p<0.001$). Three patients valued transfusion dependence as worse than being dead. Corresponding rating scale scores were 78 vs. 57 ($p<0.001$), and 78 vs. 32 ($p<0.001$), respectively. *Conclusions.* These results show that patients associate a high value with achieving transfusion independence, which, in turn, suggests an important role for new treatments aiming to achieve greater transfusion independence in MDS.

0809

HEALTH RELATED QUALITY OF LIFE IS COMPROMISED IN PATIENTS WITH IRON OVERLOAD RECEIVING INFUSION TREATMENT: US AND UK SF-36 AND CHQ SCORES COMPARED TO MATCHED POPULATION NORMS

D. Rofail,[1] M. Viala,[2] E. Trudeau,[2] J.F. Baladi,[3] K.A. Payne[4]

[1]*Mapi Values Ltd, Bollington, United Kingdom;* [2]*Mapi Values France, Lyon, France;* [3]*Novartis Pharmaceuticals Corporation, New Jersey, USA;* [4]*Caro Research Institute, Quebec, Canada*

Backgrounds. Patients with thalassemia, sickle cell disease (SCD), and myelodysplastic syndrome (MDS) require blood transfusions as supportive care. One consequence of regular blood transfusions is iron overload (IO) which, if left untreated, will result in morbidity and earlier mortality. Current infusion iron chelation therapy (ICT) requires 8-12 hour infusions, 5-7 days per week, potentially inhibiting adherence and limiting health-related quality of life (QoL) in patients already limited by thalassemia, SCD, and MDS. *Aims.* To assess the HRQoL of patients on infusion ICT by comparing the Medical Outcomes Study Short Form Health Survey (SF-36) and Child Health Questionnaire (CHQ) responses from individuals with thalassemia, SCD, and MDS, undergoing infusion ICT against US and UK norms. *Methods.* Patients with TLA, SCD,

or MDS, currently undergoing infusion ICT, completed the SF-36 and CHQ. In total 79 participants were assessed (US: thalassemia n=41 and SCD n=9; UK: thalassemia, n=11 SCD n=14, and MDS n=4). The HRQoL instruments, the SF-36 and CHQ, were scored (0 to 100, with higher scores indicating higher QoL) and compared to available age- and/or gender- matched published norms for UK and US. In addition, utilities were estimated for the UK population using published algorithms developed by Brazier *et al*. 2001 to transform SF-36 scores into utility-based scores. *Results.* In the US, compared to age- matched norms, study participants scored lower on all SF-36 domains (decremental point difference ranged from 0.2 for Mental Health to 23.95 for General Health), except for Role Emotional (incremental point difference of 2.61). In the UK compared to age- and gender matched norms, study participants scored lower on all SF-36 scales. Specifically, point differences between UK male norms and UK male study population indicated a decrement ranging from 1.4 for the Mental Health domain to 61.25 for Role Performance domain. Similar results were reflected in the female group in whom, compared to UK norms, point differences were lower and ranged from 5.03 for Mental Health domain to 56.73 points in Role Performance. CHQ data revealed similar results. In the US, compared to age-matched norms, study participants scored lower on all CHQ scales (decremental differences ranged from 2.08 for Physical Functioning to 26.64 for Parent-Impact Emotional) except Family Cohesion, General Behaviour, and Self-Esteem. In the UK, compared to age-matched norms, study participants scored lower on all CHQ scales (reduced point difference ranged from 1.65 for Self Esteem to 33.03 for Parent-Impact Emotional) except Family Cohesion. Differences of these magnitudes are generally considered clinically significant. Further, UK study participants produced a utility score of 0.61. *Summary/Conclusions.* Results indicated that patients with thalassemia, SCD, or MDS currently undergoing infusion ICT showed much lower HRQoL scores compared to population norms, and particularly for General Health, Role Physical, and Parent-Impact Emotional. Reducing the burden of treatment on the patient by having an effective, well tolerated, and more convenient therapy would contribute to improving the quality of life of these patients.

0810

ESTIMATED TOTAL ANNUAL COSTS OF INFUSED IRON CHELATION THERAPY IN THE UNITED KINGDOM

M.P. Desrosiers,[1] K. Payne,[1] I. Proskorovsky,[1] K. Ishak,[1] J.F. Baladi[2]

[1]*Caro Research Institute, Montreal, Canada;* [2]*Novartis Pharmaceuticals Corporation, Florham Park, USA*

Backgrounds. Patients suffering from β-thalassemia, sickle cell disease, and myelodysplastic syndrome undergoing chronic blood transfusions are at risk for iron overload which, if not treated by iron chelation therapy (ICT), can create serious organ damage and reduce life expectancy. Deferoxamine (DFO) is the standard of care for the depletion of excess bodily iron. It has to be infused for 8-10 hours, 5-7 times a week. Although the clinical need for ICT is clearly established, less is known about the economic burden of DFO treatment. *Aim*. To estimate the total annual costs of DFO ICT from patient chart reviews, resource utilization in UK treatment centers and the medical literature. *Methods.* A retrospective, prospective study of ICT was conducted in 29 patients (11 β-thalassemia; 14 sickle cell disease; 4 myelodysplastic syndrome; 31% male; mean age 30.6 ± 20.1 years) from four UK treatment centers on the basis of chart reviews and interviews. Data related to prescribed ICT regimens, infusions performed in a health care setting, monitoring, and treatment of adverse events from the patient's initial and most recent year of ICT were abstracted from the charts. In an interview, patients were queried about their compliance to ICT, as well as resource utilization related to ICT equipment, supplies, home delivery and ICT adverse events over the previous 30 days. To supplement data from the study, a review of the literature was performed. Unit costs (2004/2005 GBP) were applied. *Results.* For all patients, the estimated mean weighted annual cost of infusions performed in a health care setting, ICT home delivery, equipment, monitoring, treatment of infused ICT-related adverse events and home health care were: £1,470; £274; £7,367; £503; £156 and £4,067 respectively. The estimated annual per patient mean cost of the drug alone, adjusted for compliance, ranged from £2,067 to £4,259, depending on the age and weight of the patient (mean weighted annual cost: £3,384). Thus, total mean per patient treatment costs were estimated to range from £11,837 to £18,096 (mean weighted annual cost: £13,154). When only ancillary costs were considered, the mean weighted cost per infusion was estimated to be as high as £70, including home health care costs. When home health care was excluded, the mean weighted ancillary cost per infusion was estimated to be £50. The total annual costs of DFO ICT were underestimated given that treatment costs for the clinical consequences of poor adherence to DFO and lost productivity were not included. *Summary/Conclusions.* Total costs of ICT appear substantial and well exceed the cost of DFO alone. The drug cost accounts for only 13% to 30% of annual treatment costs of DFO treatment (26% of total mean weighted annual cost).

0811

SAFETY, EFFICACY AND COST-EFFECTIVENESS OF REGULAR PROPHYLAXIS WITH REFACTO IN ADULTS WITH SEVERE HEMOPHILIA A: A PROSPECTIVE STUDY

A. Gringeri,[1] M. Monzini,[2] S. Ravera,[3] C. Santoro,[4] R. Musso,[5] L. Mantovani[6]

[1]*University of Milan, Milan, Italy;* [2]*Angelo Bianchi Bonomi Haemophilia and Th, Milan, Italy;* [3]*Centre of Pharmacoeconomics, Department, Milan, Italy;* [4]*Department of Haematology, La Sapienza U, Rome, Italy;* [5]*Haemophilia Centre, Department of Haemat, Catania, Italy;* [6]*CIRF/Centre of Pharmacoeconomics, Facult, Naples, Italy*

Objectives. No prospective study is available to evaluate efficacy and cost-effectiveness of prophylaxis carried out in adults with hemophilia. Moreover, prophylaxis with a B-domain-deleted recombinant FVIII (ReFacto™) has been reported by some Authors to have poor efficacy and risk of inhibitors. This prospective study was designed to evaluate safety, efficacy and cost of prophylaxis with Refacto™ in adults with hemophilia. Secondary aim is the evaluation of the health-related quality of life (HRQoL) of patients on prophylaxis compared to patients on on-demand treatment. *Methods*. We enrolled patients with severe hemophilia A, age ≥18 years, at least 18 bleeds in the previous year, switching from on-demand treatment with a plasma-derived FVIII to prophylaxis, with 25 IU/Kg 3 times a week (tiw) of Refacto™. Bleeds, concentrate consumption and HRQoL were evaluated in the 6-month on-demand period (ODP) before enrolment, compared to the following 6-months prophylaxis period (PP). Medical costs were quantified from the National Health Service's perspective. Two generic HRQoL questionnaires, EuroQol (EQ-5D) and Short Form 36 (SF-36)) have been used, higher score corresponding to better quality of life. *Results.* None of the 19 enrolled patients, aged 23-58 years (mean=33.2) developed inhibitors. Patients reported a mean of 2.97 events/patient/month during ODP (median=1.67, range: 0.5-15) vs. 0.48 events/patient/month during PP (median=0.25, range: 0-2.5) (Wilcoxon signed-test $p<0.001$). Mean FVIII consumption was 16,149 IU/patient/month during ODP (range: 2,000-50,000) and 26,342 IU/patient/month during PP (range: 16,667-38,333). Mean FVIII cost during ODP was 9,090 /patient/month (range: 968-27,300) vs. 20,732 /patient/month (range=13,583-29,095) during PP. The mean cost to treat one bleed was 3,958 (median: 2,391). The incremental cost-effectiveness ratio, i.e. the cost for avoided bleed, was 4,675, At the end of the follow-up period, SF-36 showed a statistically significant improvement in patients quality of life in all domains ($p<0.05$). Concerning the Physical Component Summary score (PCS) and the Mental Component Summary score (MCS), patients on PP showed better results than those on ODP, although no significant difference was found for MCS. Results obtained with EuroQol-5D were comparable to those showed by SF-36, with significantly different Visual Analogue Scale scores after ODP vs. after PP(63.06 and 72.65 respectively, $p<0.01$). *Summary/Conclusions*. These findings showed prophylaxis with Refacto™'s dose of 25 IU/Kg tiw in adults with haemophilia was effective and safe. Our cost-effectiveness results can represent the point of reference for other similar evaluations. Furthermore prophylaxis has provided a significant improvement in HRQoL and it should therefore be considered in a cost utility evaluation.

0812

COST UTILITY ANALYSIS OF DEFERASIROX VERSUS DEFEROXAMINE FOR PATIENTS REQUIRING IRON CHELATION THERAPY IN THE UNITED KINGDOM

J. Karnon,[1] R.L. Akehurst,[1] N.L. Papo[2]

[1]*University of Sheffield, SHEFFIELD, United Kingdom;* [2]*Novartis Pharmaceuticals UK Ltd, CAMBERLEY, United Kingdom*

Backgrounds. Patients suffering from β-thalassemia (β-thal), sickle cell disease (SCD), and myelodysplastic syndrome (MDS) require lifelong blood transfusions and are at risk of iron overload. If they are not treated with iron chelation therapy (ICT), they can suffer serious organ damage and reduced life expectancy. Desferal, infused subcutaneously for 8 to 10 hours per day, 5 to 7 times per week, is the standard of care for the depletion of excess bodily iron. Exjade is a new once daily oral iron

chelator, which has recently been approved by the FDA and Swissmedic for the treatment of transfusional iron overload. *Aim*: To estimate the incremental cost per quality adjusted life year (QALY) of using Exjade instead of Desferal in patients with β-thal, SCD, or MDS who require iron chelation, from a UK NHS perspective.

Table 1.

Both case	*Desferal*	*Exjade*	*Difference*
Drug costs	£ 3511	£ 11520	£ 7739
Administration costs	£ 7551	£ 0	-£ 7551
Total costs	£ 11063	£ 11250	£ 187
Utility value	0.61	0.85	0.24
Incremental cost per QALY			£ 779

Sensitivity analysis	*Difference in costs*	*Difference in costs*	*Incremental cost per QALY*
Mean weight increased to 70 kg	£2408	0.24	£ 10333
Decreased Exjade dose to 10 mg/kg	-£ 5438	0.24	Exjade dominates
Increase Exjade dose to 30 mg/kg	£ 5812	0.24	£ 24217
Decreased utility gain by 50%	£ 187	0.12	£ 1559

Methods. An aggregate annual cost of ICT with Desferal was informed by a primary study of 29 patients (11 β-thal; 14 SCD; 4 MDS; 31% male; mean age 30.6 ± 20.1 years, mean weight 54kg) from four UK treatment centers on the basis of chart reviews and interviews. Major resource items included drug costs, home delivery pumps and balloon infusors, and items relating to the use of portacaths. For Desferal, weighted mean prescribed annual dose frequency was 236, with a compliance rate of 83.7%; mean dosage was 37 mg/kg at a cost of £8.88/g. Exjade was assumed to have a prescribed frequency of 365 doses per year, with the same compliance rate as observed for Desferal (83.7%), and a dose of 20 mg/kg at a cost of £34/g. Unit costs (2004/2005 GBP) were applied. Costs related to monitoring were excluded, as were adverse events as a conservative assumption of equal compliance with Desferal and Exjade was defined. Annual utility values reflecting the impact of subcutaneous infusion compared with oral administration were estimated to be 0.61 and 0.85, respectively (*Lourenco et al., ASH 2005*). *Results.* In the base case analysis, Exjade has an extremely low incremental cost per QALY of £779, as shown in the *Results*. see Table. A range of one-way sensitivity analyses are also presented. *Conclusions.* The once a day orally administered Exjade appears to offer an extremely cost-effective alternative option to the current infusion-based iron chelator Desferal.

0813

COST-EFFECTIVENESS OF ALEMTUZUMAB IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WHO HAVE FAILED ALKYLATING AGENTS AND FLUDARABINE

S.T. Thompson,[1] F.N. van Nooten,[2] M.A. van Agthoven,[3] P.H. Huijgens[4]

[1]*Schering AG, Berlin, Germany;* [2]*MC - University Medical Centre, Rotterdam, Netherlands;* [3]*Erasmus MC University Medical Centre, Rotterdam, Netherlands;* [4]*VU University Medical Centre, Amsterdam, Netherlands*

Backgrounds. Currently, there are no cost-effectiveness data available comparing the use of alemtuzumab (MabCampath™) with alternative therapies for relapsed/refractory CLL after alkylating agents and fludarabine, ie, *third-line* patients. AIM: The objective of this analysis was to estimate the cost-effectiveness of 8 weeks of alemtuzumab administered intravenously (IV) or subcutaneously (SC) when compared with 1) an alternative antibody: high-dose rituximab, 2) 6 cycles of CHOP, the old standard. *Methods.* The effectiveness evidence used for analysis was derived from a review of published clinical studies enrolling patients who failed fludarabine, except in the case of CHOP, where the limited data available were mostly based on patients with less advanced disease. 'Expected months in remission per patient treated' was used to assess the health benefit of each therapy, and was estimated by multiplying the weighted overall response rate by the duration of response. In addition, for comparison between alemtuzumab and CHOP, life-months gained were calculated. Resource use associated with each intervention was based on the published literature, a previous patient level costing study conducted in the Netherlands in follicular lymphoma, and expert opinion. Unit costs for the Netherlands were derived from local hospital accounting systems, published tariffs, and listed wholesale prices. Sensitivity analysis was used to test the robustness of the findings. Unit costs were based on the 2003 price year. *Results.* Mean total cost of treatment with alemtuzumab IV was calculated to be approximately €23,281. Savings associated with a switch from IV to SC alemtuzumab are €3,035 with hematologist administration and higher with self-administration. Although cost of treatment with CHOP, €7,174, is lower than with alemtuzumab, in terms of health benefit the expected number of months in remission per patient treated is 3.61 months with alemtuzumab versus 1.59 months for CHOP. Cost of 12-dose rituximab, €30,155, is higher than alemtuzumab, and the expected time in remission is less at 1.98 months. Comparison of health benefits for each therapy with their cost shows that the mean costs per month in remission for alemtuzumab and CHOP are within a similar range: CHOP is €4,519 as base-case scenario, alemtuzumab SC 5,608, and alemtuzumab IV €6,449. For 12-dose rituximab the cost per month in remission is considerably higher at €15,195. When compared with a historical chemotherapy control, alemtuzumab is associated with a survival gain of approximately 8 months. Assuming this level of health benefit over CHOP would lead to an incremental cost per life-year gained for alemtuzumab IV of 24,160. *Conclusions.* This analysis shows that for each *third-line* CLL treatment where alemtuzumab IV is used instead of CHOP, additional cost to the payer would be on average €16,107, or less at 13,072 for SC administration by a hematologist. Benefit to the patient would be 8 months of survival gain, on average. The associated incremental cost per life-year gained for alemtuzumab IV over CHOP would be €24,160, well within the accepted range. Furthermore, when the costs and benefits of alemtuzumab are compared with high-dose rituximab monotherapy, alemtuzumab is both less expensive and more effective.

0814

A QUALITY MANAGEMENT SYSTEM FOR JACIE ACCREDITATION AT MINIMAL FINANCIAL COST

S. van Steenweghen,[1] A. Gadisseur,[2] A. Van de Velde,[2] E. Steel,[2] I. Vrelust,[2] K. Van Brussel,[2] Z. Berneman,[2] W. Schroyens[2]

[1]*Universitair Ziekenhuis Antwerpen, EDEGEM, Belgium;* [2]*University Hospital Antwerp, EDEGEM, Belgium*

Backgrounds. The Joint Accreditation Committee of the International Society for Cellular Therapy and the European Blood and Marrow Transplantation Group (JACIE) offers an accreditation programme to transplant centers that are willing to benchmark and optimize their transplant activity on a voluntary basis. As a major practical challenge and change in day-to-day patient care, submission to this programme demands documentation of education and training, reporting of clinical outcomes and the formalisation of a Quality Management System (QMS). Commercial QMS solutions are readily available, but these increase the financial burden of the application for accreditation considerably. *Aims.* Materialize a QMS at minimal financial cost. *Methods.* We used open source software to address the need for event reporting which is an essential part of a QMS. Open source software prevents vendor lock-in and guarantees permanent open data availability with adjustable security levels. Legally, this type of software carries the General Public Licence (GPL). The licence is intended to preserve the freedom to share and change free software. For this purpose the source code is always available. We used as software packages Apache (hypertext markup language webserver), PHP (hypertext preprocessor) and Mysql (relational database). This allowed us to deliver, using the local intranet, a front end to an eventreporting database back end, and this with minimal developmental efforts and minimal cost. Development of this tool consisted of language adaptation of the PHP scripts, which are shared thanks to HOT (HOT-Open Tickets): a user-friendly open source trouble-ticket system. This stable system delivers as main functions : the ordering of events by reporter and subject, the reminding of collaborators of the state of the event, the guiding of resolution and the delivering of reports for internal audits. These reports provide essential registration items for further follow up and can also be used for hospitalwide or nationwide clinical event reporting. The convenience of the front end ensures a low threshold to report events to the busy but willing field workers. The possibility reporting anonymously but with assignment of an issue manager who follows the event until solution, further decreases the likeli-

hood that an event with relevance to quality enhancement will pass unreported. The software is free of charge, and installation takes around 15 hours of paid collaborator time. The solution can be shared on request, conform GPL. It is extendable for documenting educational JACIE needs. Screenshots will be presented. *Results and conclusion.* A tool for event reporting was established at minimal cost through open source software. It guarantees indefinite availability of data and anticipates future legislation. It contributes to a quality management system as needed for JACIE accreditation.

Links

http://www.gnu.org
http://www.apache.org
http://www.php.net
http://hotopentickets.sourceforge.net
http://www.jacie.org

0815

ESSENTIAL THROMBOCYTHEMIA: ANAGRELIDE OR INTERFERON-ALPHA A COST-UTILITY ANALYSIS

G. Froyen,[1] R. Fordham[2]

[1]*VIB, Leuven Belgium;* [2]*University of East Anglia, Norwich, United Kingdom*

Backgrounds. Essential thrombocythaemia (ET) is a rare myeloproliferative disorder characterised by an abnormally high platelet count, leading to increased risk of thrombohaemorrhagic events. Platelet reduction therapy is indicated in high-risk patients, with hydroxycarbamide used as first-line treatment. However, up to 20% of patients are refractory or intolerant, and therefore require a second-line alternative. Busulfan, radiophosphorus (32P) and pipobroman are rarely used owing to their leukaemogenic potential. Interferon α (IFN-α) and anagrelide are two platelet-lowering agents thought not to possess this same risk. As resources for healthcare expenditure are finite, it is desirable to know which of these two options provides the best value. *Aims.* To assess the relative cost-effectiveness of IFN-α and anagrelide as second-line therapy for the management of ET in high-risk patients. *Methods.* A Markov model, capturing the risk of deep vein thrombosis (DVT), cardiac, stroke, and gastrointestinal (GI) bleed events, was developed to estimate the lifetime costs and quality-adjusted life-years (QALYs) gained from treating a notional 60-year-old female with anagrelide or IFN-α as second-line ET therapy. All data used to populate the model were extracted from the published literature. *Results.* Anagrelide as a second-line treatment for ET results in improved overall quality of life relative to IFN-α (12.35 versus 11.47 QALYs). The lifetime cost of anagrelide is higher than for IFN-α (£58,624 versus £45,702 [costs and benefits discounted at 3.5%]). Therefore, the extra cost per QALY gained from anagrelide compared with IFN-α is £14,805. This result is sensitive to assumptions on the impact of treatment side-effects and subcutaneous injections on quality of life. However, taking into account uncertainty within the input parameters, we estimate that for 89.5% of patients, the incremental cost-effectiveness ratio (ICER) will be below £30,000, the typical willingness-to-pay threshold for a QALY in the UK National Health Service. *Conclusion.* For high-risk patients suffering from ET, anagrelide is likely to be a more cost-effective second-line option compared with IFN-α.

Allogeneic stem cell transplantation II

0816

IMMUNOLOGIC RECOVERY AND GRAFT VERSUS HOST DISEASE AFTER NONMYELOABLATIVE STEM CELL TRANSPLANTATIONS

J. Bennani,[1] N. Meuleman,[1] P. Lewalle,[1] P. Martiat,[1] A.I. Ahmad[1], M. Aoun,[1] W. Ferremans,[2] R. Leroy,[1] M. Paesmans,[1] D. Bron[1]

[1]*Jules Bordet Institute, Brussels, Belgium;* [2]*Erasme Hospital, Brussels, Belgium*

Background. Non myeloablative stem cell transplant (NSCT) is an important therapeutical option for those patients (pts) who are not eligible to conventional transplant The curative potential of these transplants are directly related to the anti tumor effect of the graft and rapidity of donor T cells engraftment. *Methods*. We have thus investigated the cellular immunologic recovery in 30 pts (20-64 years old) given peripheral blood stem cells from Matched Related and Unrelated Donors, mostly receiving a conditioning regimen based on fludarabine, Antithymocyte globulin (ATG) and Cyclophosphamid (or cytarabine or melphalan). Post grafting immunosuppression usually consisted of cyclosporin and mycophenolate mofetil. The mononuclear blood cell subsets were assessed by flow cytometry; analyses were performed on days 30, 60, 90, 180 and 360 after SCT. Incidence of graft versus host disease (GVHD) and opportunistic infections was correlated with immune recovery. Results. We observed an early recovery of $CD8^+$ T cell (d8) and NK cells (d22) whereas $CD4^+$ T cells remain below the normal value until d360. This slow $CD4^+$ T cell recovery was not correlated with the total T cell number in the graft in our series. GVHD rate was similar to classical SCT -maybe with a lower severity- but mostly delayed compared to classical SCT. Our data show that a low rate of $CD4^+$ T cells does not protect from GVHD, but this delay in T cell recovery might explain the late occurrence of GVHD in NSCT and also a later CMV-specific immune reconstitution translated into an increased frequency of CMV-reactivations. However, this did not lead to increased CMV diseases. We also observed a higher rate of CMV infections when $CD4^+$ T cells were below 100/μL. Invasive fungal infections were not correlated with $CD4^+$ T cells recovery in our study and mostly observed in patients receiving steroids for GVHD. 80% of patients in complete response after NSCT had developed a GVHD. Donor lymphocytes infusion was mostly useful to salvage relapsing who failed to present a GVHD after SCT. *Conclusions*. Our small series confirms the good tolerance of these NSCT, a similar immune reconstitution pattern to those seen after myeloablative SCT, a late and low rate occurrence of GVHD using ATG in the conditioning regimen, and an increased rate of CMV infections correlated with a low count of $CD4^+$ T cells.

0817

LOW-DOSE METHOTREXATE AS SALVAGE THERAPY FOR REFRACTORY GRAFT-VERSUS-HOST DISEASE AFTER REDUCED-INTENSITY CONDITIONING FOR ALLOGENEIC STEM CELL TRANSPLANTATION

M. Mohty, H. De Lavallade, C. Faucher, S. Furst, J. El Cheikh, D. Blaise

Institut Paoli-Calmettes, Marseille, France

Corticosteroid (CS)-resistant GVHD is associated with high morbidity and mortality, and therapeutic options are limited for those patients. Also, elderly patients undergoing RIC allo-SCT are more exposed to the side effects of long term CS administration. Low dose methotrexate (MTX) therapy is a well established modality for GVHD prophylaxis. However, little is known about the role of this drug in CS-resistant GVHD. This pilot single center study investigated the role of MTX in a curative setting after failure of CS treatment in patients undergoing RIC allo-SCT. 20 patients received IV infusions of low dose MTX (5 mg/m2/infusion) at weekly intervals, for at least 4 weeks. Reasons for MTX administration were: CS-refractory acute GVHD, CS-refractory chronic GVHD, chronic GVHD exacerbation after CS taper, or CS severe side effects. Responses to low dose MTX infusions were assessed one month after the last infusion in each involved organ. 12 patients were treated for severe acute GVHD, while 8 patients received MTX for extensive chronic GVHD. Median age of patients was 51 (range, 22-60). Median time of administration of MTX was day +89 (range, 32-300). Of note, none of the patients received any other concomitant therapy for refractory GVHD. 13 patients responded to MTX administration (65%) with 5 complete responses (25%). Among the 12 patients treated for acute GVHD, 7 responded (58%) of whom 5 CRs (42%). 3 patients did not respond and died from resistant GVHD. Interestingly, 5 patients

from the group of grade 3-4 acute GVHD responded. Among the 8 patients treated for chronic GVHD, 6 were responders (75%). In addition, MTX allowed a significant reduction of CS daily dosage ranging from 25% to 80%, as assessed one month after the last administration of MTX. With a median follow-up of 287 days, no increase of CS therapy was necessary among these 6 MTX-responding patients. In all, toxicity of low dose MTX administration was low (transient and mild reversible cytopenia in 3 cases, 15%). Among the 20 patients, 14 are still alive (70%) with a median follow-up of 293 (range, 65-513) days. Overall, 2 patients died of progressive disease, while 4 patients died from refractory GVHD. We conclude that low dose MTX is a well-tolerated, inexpensive and likely steroid-sparing agent that is worthy of further investigation in prospective trials for treatment of refractory GVHD, but also as frontline therapy in combination with CS.

0818

OBSERVATION-BASED EARLY WARNING SCORES TO DETECT IMPENDING CRITICAL ILLNESS PREDICT IN-HOSPITAL AND OVERALL SURVIVAL IN PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION

M. von Lilienfeld-Toal,[1] K. Midgley,[2] S. Lieberbach,[2] L. Barnard,[2] A. Glasmacher,[1] M. Gilleece,[2] G. Cook[2]

[1]*Uniklinik Bonn, BONN, Germany;* [2]*BMTU, St. Jamess University Hospital, Leeds, United Kingdom*

Backgrounds. Observation-based early warning scoring systems have been developed to improve the outcome of critically ill patients by triggering early critical care intervention. To date none of these scoring systems have been validated in cancer patients or stem-cell transplant recipients. *Aims.* The aim of this study was to validate three established scoring systems in adult recipients of Allogeneic stem cell transplantation (Allo-SCT) and to determine their usefulness at predicting survival. *Methods.* We retrospectively analysed the physiological observations during the initial admission of patients undergoing Allo-SCT. Three different early warning scoring systems termed MEWS, PARS and LEWS (Table 1) were assessed.

Table 1. Leeds based modified early warning score (LEWS).

Score	*3*	*2*	*1*	*0*	*1*	*2*	*3*
Heart Rate (beats/min)		<40	41-50	51-100	101-110	111-130	>130
Systolic blood pressure (mmHg)	<70	70-80	81-100	101-179	180-199	200-220	>220
Respiratory rate (min-1)		<8	8-11	12-20	21-25	26-30	>30
Oxigen saturation (%)	<85	85-89	90-94	≥95			
Respiratory support	BiPAP/ CPAP	Hi/ Flow	Oxygen Therapy				
Urine output in last 4 hours	<80	80-120	121-200	201-799	>800		
Level of consciousness			Confusion	Alert	Reacts to voice	Reacts to pain	Unresponsive

Results. Charts of 43 patients (AL n=21, HD/NHL n=10, MM n=4, CML n=7, SAA n=1) with a median age of 40 years (IQR 29-49) were analysed. 29 of 43 patients received grafts from matched sibling donors and 18 of 43 received radiation-based full intensity conditioning. Impairment of respiratory function was the commonest (40 patients, 93%) event, usually deteriorating during the second week post-graft. All scores revealed high accuracy in predicting in-hospital survival (AUC in ROCC for MEWS 0.915, for PARS 0.938 and for LEWS 0.988 respectively, $p < 0.001$). For all three scores the cut-off level associated with a high risk of in-hospital mortality was 7. Of eight patients with a LEW score of ≥7, 7 died during their initial admission, whereas no patient with a lower score died (PPV 88%, NPV 100%, specificity 97%, sensitivity 100%). Acute clinical deterioration during the initial admission appeared to have an adverse effect on overall survival after discharge: in-hospital survivors who reached a LEW score >3 during their admission had a median survival of 663 days (median survival not reached in patients with LEWS < 3, p=0.018). *Conclusions.* This is the first study to validate early warning scoring systems in Allo-SCT and demonstrate that these systems are highly predictive of in-hospital survival and overall survival post-discharge. The most likely time to develop clinical deterioration is the second week after graft infusion.

0819

A DOSE FINDING STUDY OF IV BUSULFAN IN COMBINATION WITH CYCLOPHOSPHAMIDE AS CONDITIONING REGIMEN PRIOR ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDREN WITH MALIGNANT AND NON-MALIGNANT HAEMATOLOGICAL DISEASES: OPTIMISATION OF BUSULFAN TREATMENT

H. Esperou,[1] G. Vassal,[2] F. Méchinaud,[4] C. Galambrun,[3] G. Michel,[5] B. Neven,[6] K. Yacouben,[7] P. Bordigoni,[8] C. Paillard,[4] H. Zouabi,[9] C. Levrault,[9] L. Nguyen,[9] V. Cadic[9]

[1]*Hopital Saint Louis, PARIS, France;* [2]*Institut Gustave Roussy, VILLEJUIF, France;* [3]*Hopital Debrousse, Lyon, France,* [4]*Hopital Hotel Dieu, NANTES, France;* [5]*Hopital la Timone, MARSEILLE, France;* [6]*Hopital Necker-Enfants Malades, PARIS, France;* [7]*Hopital Robert Debré, PARIS, France;* [8]*Hopital d'Enfants Brabois, VANDOEUVRE LES NANCY, France;* [9]*Institut de Recherche Pierre Fabre, BOULOGNE BILLANCOURT, France*

Backgrounds. In pediatric patients (pts) oral busulfan (Bu) is often included in conditioning regimens prior to allogeneic (allo-) haematopoietic stem cell transplantation (HSCT). Bu has a narrow therapeutic window and under- or overdosing may have a fatal outcome. Bu clearance (Cl) is high in children and higher doses are needed to obtain an area under the curve (AUC) equivalent to adults. To optimise Bu treatment we defined (Nguyen L. et al BMT 2004) and assessed prospectively a new IVBu fixed dosing allowing to 91% of 55 pts targeting AUC (900-1500 µM.min) without therapeutic drug monitoring (TDM) (*Pharmacokinetic results were reported separately by G Vassal et al.*). We report here the clinical outcome of these children after allo-transplant. *Aims.* To investigate the safety of this new IVBu dosing strategy, to assess whether theses doses are myeloablative and supportive for engraftment, and to evaluate the consequences of IVBu dosage upon children clinical outcome Methods. Children (15 male/13 female) received IVBu-based Bu-cyclophosphamide (Cy, 200mg/kg) prior to HSCT. IVBu (over 2 h infusion) was given based on body weight: 1.0 mg/kg, 1.2 mg/kg, 1.1 mg/kg, 0.95 mg/kg, and 0.8 mg/kg for pts with <9 kg, 9 to <16 kg, 16-23 kg, >23-34 kg, and >34 kg strata of weight, respectively. Clonazepam was given as seizures prophylaxis. Indications for HSCT were: AML (n = 14, 12 CR1, 1 CR2, 1 PR), CML (n = 3), ALL (n = 1), MDS (n = 1); hemoglobinopathy (n = 6), and immunodeficiency (n = 3). Recipients aged from 0.3 to 17.2 years (median 7.2 y) received bone marrow containing 5.7×10^6/kg $CD34^+$ (range 1.1-28) from matched related (22/28) or unrelated (4/28) donors. Regimen-related toxicity (RRT) was graded according to NCI-CTC 2.0. Kaplan-Meier EFS and OS were evaluated. Results. All had profound myeloablation, and all (28/28, 100%) engrafted at 21 days (range 12-47) for neutrophils > 0.5×10^9/L, and 33 days (range 16-90) for platelets > 50.0×10^9/L. Donor cells were documented in all recipients: 25/28 achieved complete chimerism (CC, > 99% donor), and 3/28 were mixed chimeras but had mainly donor cells (> 85%). No primary and/or secondary graft rejection occurred. IVBu was well tolerated: no grade (G) IV toxicity, G III 14 pts (mainly stomatitis), and G I-II 24 pts. VOD incidence was 7% (95% CI: 0.9-23.5%) but none was severe. Grades I-II and-III-IV a-GVHD rates were 46% and 4%, respectively. There was no death at day+100, and Cumulative TRM incidence at 2 years was 4%. Four pts died due to c-GVHD (n = 1), AML relapse (n = 3). After a median follow-up of 28.0 months (range 18.2-38.2) estimated EFS and OS rates were 85% + 14% for both probabilities. *Summary/Conclusions.* These results indicate that this new IV Bu dosing optimises allo- engraftment with CC in 90% of pts, is well tolerated resulted in a very weak TRM, which translated into promising survival.

0820

DACLIZUMAB HAS POOR EFFICACY IN STEROID-REFRACTORY ACUTE GRAFT VERSUS HOST DISEASE: A SINGLE CENTRE EXPERIENCE WITH 12 ALLOGRAFT PATIENTS

H. Sia, C.H. Hui, H. Mangos, N. Horvath, H.K. Lee, I. Lewis, T. Hughes, B. To, P. Bardy

Royal Adelaide Hospital, Adelaide, Australia

Backgrounds. Daclizumab, a humanised monoclonal antibody against interleukin-2 receptor, has been used in steroid-refractory acute graft-versus-host disease (aGVHD). Reported results were conflicting. Aims and Methods. We performed a retrospective audit of the outcome data of 12 consecutive allograft patients who had been treated with Daclizumab for steroid-refractory (to > 2mg/kg/d of iv Methylprednisolone) grade III-IV aGVHD from year 2000-2004 in our unit. All patients received standard anti-microbial prophylaxis, and Cyclosporin and Methotrexate GVHD prophylaxis, except for three reduced-intensity allografts who received cyclosporine alone. Clinical grading of aGVHD was performed according to standard criteria. 1mg/kg of iv Daclizumab was given on days 1, 4, 8, 15 and 22 and definition of treatment response as previously described (*Przepiorka 2000*). *Results.* Twelve patients developed grade III-IV aGVHD after HLA-matched blood stem-cell allogeneic transplants, who consisted of 9 sibling (7 ablative, 2 reduced-intensity) and 3 unrelated (1 ablative, 2 reduced-intensity) allografts. Daclizumab was commenced after failure of iv Methylprednisolone at a median of 81/2 days (range: 3-28). These patients also received numerous (range: 3-7) concomitant GVHD therapies, including Steroids, Cyclosporin/Tacrolimus, Mycophenolate, and Etanercept (for gut aGvHD). Anti-thymocyte globulin (ATG) was additionally given for poor responders to Daclizumab in 6/12 patients. The only complete responder to Daclizumab (patient 7) died of subsequent exacerbation of gut GVHD, haemorrhagic cystitis and CMV viraemia. The single partial responder (patient 6) eventually died of progressive gut GVHD and bacterial sepsis. There was no long-term survivor with infections as terminal events in 10/12 patients. *Conclusions.* In contrast to initial published reports, allograft patients with severe steroid-refractory aGVHD had poor response and dismal outcome when treated with Daclizumab in our institution. It was our major concern that the poor survival may be contributed by the delay of more appropriate GVHD therapy and the aggravation of infective complications. As a result, we have moved away from Daclizumab back to ATG since 2005. Novel GvHD therapies such as photopheresis, mesenchymal stem cells should be explored.

Table 1. Pre- and post-Daclizumab responses and outcome.

	Skin GVHD		Gut GVHD		Liver GVHD		
Score	Pre-	Day 43	Pre-	Day 43	Pre-	Day 43	Outcome
1	0	0	1	2	3	4	Death, d60
2	3	3	2	2	1	3	Death, d57
3	3	2	2	3	0	4	Death, d124
4	3	3	3	4	0	1	Death, d180
5	0	0	0	0	4	4	Death, d31
6	1	0	3	1	2	1	Death, d345
7	0	0	2	0	2	0	Death, d323
8	0	0	0	0	4	4	Death, d240
9	1	1	3	4	2	4	Death, d247
10	0	0	3	4	0	0	Death, d72
11	1	0	3	4	2	4	Death, d53
12	2	1	3	1	3	4	Death, d166

0821

ALLOGENEIC STEM CELL TRANSPLANTATIONS AFTER REDUCED INTENSITY CONDITIONING REGIMEN FLUDARABIN, BUSULFAN AND ATG (FRESENIUS)

Y. Brychtova,[1] M. Doubek,[1] J. Muk,[2] J. Mayer,[1] J Vorlcek[1]

[1]*University Hospital Brno, Brno, Czech Republic;* [2]*Centre Biostatistics Masaryk University, Brno, Czech Republic*

Introduction: Allogeneic stem cell transplantation with reduced intensity conditioning (RIC) is effective therapy for hematologiccl diseases. Methods. This is a retrospective report about 63 patients [21 women, 42 men, median age 51 years (15-65)] who underwent hematopoietic stem cell transplantation (HSCT) after reduced intensity conditioning regimen (RIC) with Fludarabin (30mg/m2, 5 days), Busulfan (8-12 mg/kg p.o.) and ATG Fresenius (10mg/kg/d, 4 days for hematological disease in our transplant center between March 1998 and December 2005. The diagnosis were 17 AML [15 in 1st.CR, 1 in 2nd CR, 1 in relaps (R)], 3 MDS, 1 AA, 22 CML (21 in chronic phase, 1 in acceleration), 1 myelofibrosis, 3 HD (1R, 1 CR, 1 PR), 8 B-NHL (2 DLBCL, 1 FL, 3 MCL, 2 SCLL) (1R, 4 PR, 3 CR), 6 CLL (4 R, 2 PR), 2 MM (1R, 1 PR). Peripherial blood stem cells (PBSC) were used in 60 patients, bone marrow in 3 patients. Median of infused CD 34+cells was $6{,}76\times10^6$/kg. Donors were 57 related and 6 unrelated respectively. As GVHD prophylaxis, 58 patients received CsA, 2 received CsA+MTX and 3 CsA+ mycophenolat mophetil. *Results.* Median time of follow up was 25 months. After transplantation, any toxicity was observed in 25% (16) patients, 66% (42) patients developed a toxicity grade I-II, 7% (4) patients a toxicity grade III, 2% (1) patient a toxicity grade IV.

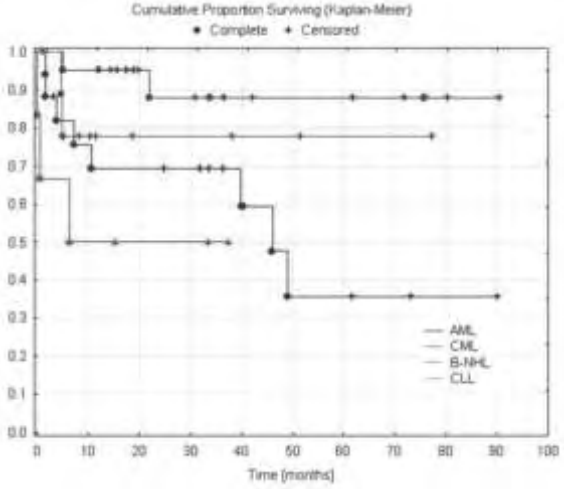


Figure. Overall survival of patients AML, CML, NHL, CLL.

Without any parenteral nutrition were 68% (43) patients, and 32% (20) patients have parenteral nutrition in median 11 days (3-22 days). Recovery of neutrofils($>1.0\times10^9$/L) was in median time 18 days, trombocytes ($>20\times10^9$/L) in median 13 days. Complete chimerism (CC) was reached in median time 73 days in 49 (78%) patients, 2 (3%) patient still didn't reach CC, 2 (3%) patients didn´t reach CC for short time after transplantation, the others didn´t reach CC because of rejection of graft (1), giving his autologous back up of stem cells for severe GVHD (1), relaps of disease (4pts=7%), death from other reason (3 infections, 1 bleeding). Twenty-two (35%) patients developed an acute GVHD: 9 patients maximal grade I, 8 patients maximal grade II, 3 patients maximal grade III, 2 patients grade IV. A chronic GVHD was presented in 28 (44%) patients (23 limited,5 extensive). Secondary rejection of graft occured in 2 patients with unrelated donor. Fourteen patients had preemptive therapy of CMV. Any infection since day +100 had 28 (44%) pts, after day +100 31 (49%) patients. Twenty patients (31%) died, the causes of death: 10 (15%) relapses of disease, 3 (5%) infections, 4 (6%) GVHD,1(2%) toxicity, 2 (3%)from other reason. Early transplant related mortality (TRM) was 10% (6 patients:2 relaps, 1 GVHD, 1 bleeding, 2 infections), late TRM 5% (3 patients GVHD). Median time of overall survival for all patients (Kaplan-Meier) wasn´t reached, AML patients had 46 months, CLL patients 6.4 months and CML patients and B-NHL patients wasn´t reached it. Conclusions. RIC is associated with favorable outcome and low toxicity in patients in remission at the time of transplantation.

0822

LOW-DOSE METHOTREXATE AS SALVAGE THERAPY FOR REFRACTORY GRAFT-VERSUS-HOST DISEASE AFTER REDUCED-INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION

H. de Lavallade, M. Mohty, C. Faucher, S. Furst, J. El Cheikh, D. Blaise

Institut Paoli-Calmettes, Marseille, France

Corticosteroid (CS)-resistant GVHD is associated with high morbidity and mortality, and therapeutic options are limited for those patients. Also, elderly patients undergoing RIC allo-SCT are more exposed to the

side effects of long term CS administration. Low dose methotrexate (MTX) therapy is a well established modality for GVHD prophylaxis. However, little is known about the role of this drug in CS-resistant GVHD. This pilot single center study investigated the role of MTX in a curative setting after failure of CS treatment in patients undergoing RIC allo-SCT. 20 patients received IV infusions of low dose MTX (5 mg/m2/infusion) at weekly intervals, for at least 4 weeks. Reasons for MTX administration were: CS-refractory acute GVHD, CS-refractory chronic GVHD, chronic GVHD exacerbation after CS taper, or CS severe side effects. Responses to low dose MTX infusions were assessed one month after the last infusion in each involved organ. 12 patients were treated for severe acute GVHD, while 8 patients received MTX for extensive chronic GVHD. Median age of patients was 51 (range, 22-60). Median time of administration of MTX was day +89 (range, 32-300). Of note, none of the patients received any other concomitant therapy for refractory GVHD. 13 patients responded to MTX administration (65%) with 6 complete responses. Among the 12 patients treated for acute GVHD, 7 responded (58%) of whom 5 CRs(42%). 3 patients did not respond and died from resistant GVHD. Interestingly, 5 patients from the group of grade 3-4 acute GVHD responded. Among the 8 patients treated for chronic GVHD, 6 were responders (75%). In addition, MTX allowed a significant reduction of CS daily dosage ranging from 25% to 80%, as assessed one month after the last administration of MTX. With a median follow-up of 287 days, no increase of CS therapy was necessary among these 6 MTX-responding patients. In all, toxicity of low dose MTX administration was low (transient and mild reversible cytopenia in 3 cases, 15%). Among the 20 patients, 12 are still alive (60%) with a median follow-up of 329 days from onset of GVHD (range, 37-633) days. Overall, 4 patients died of progressive disease, while 4 patients died from refractory GVHD. We conclude that low dose MTX is a well-tolerated, inexpensive and likely steroid-sparing agent that is worthy of further investigation in prospective trials for treatment of refractory GVHD, but also as frontline therapy in combination with CS.

0823

β THALASSEMIA MAJOR: BONE MARROW VERSUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

M. Irfan,[1] S. Adil,[2] P. Ahmed,[3] S. Ansari,[1] T. Farzana,[1] K. Hashmi,[3] B. Khan,[3] V. Panjwani,[1] T. Shamsi[1]

[1]*Bismillah Taqee Blood Disease Centre, Karachi, Pakistan;*
[2]*Aga Khan University Hospital, Karachi, Pakistan;*
[3]*Armed Forces Institute of BMT, Rawalpindi, Pakistan*

Backgrounds. Most thalassemia births are now seen in developing countries where the socioeconomic status generally remains poor. Because of low cost, donor willingness and favorable immune profile, PBSCT remains an attractive option for multiply transfused Thalassaemia patients particularly in developing countries. Aims. To compare PBSCT with BMT in Thalassemia patients in terms of rejection, GvHD & disease free survival. *Methods.* 56 patients were transplanted from September 2000 - July 2005. 29 underwent BMT and 27 received PBSCT. Most patients were intensely transfused to keep hamoglobin 13-14 gm/dl and received desferioxamine, 24 hours infusion, before transplantation. Pesaro class I (n-20) and class II (n-20) received conditioning with standard Bu/Cy. Of class III (n-16), ALG was added to standard Bu/Cy in 9 who received PBSCT and 7, who received BM, were conditioned with Hydrea 20-30 mg/kg (day - 45 to -11), Azathioprin 3 mg/kg (day - 45 to day -11), Fludarabine 25 mg / kg (day -17 to -13) followed by Bu14/Cy 200 started on day-10. Triple immunosuppression was used for PBSC group and class III. For others aGvHD prophylaxis comprised of MTX and cyclosporine only. MNC dose infused was $>4\times10^8$/kg recipient weight in PBSC patients and for BM its range was 1.6-6.8 MNC / kg . All patients received G-CSF 5mg / kg / day, from day + 5, till ANC $>0.5\times10^9$/L. Median age of the donor was 8.6 years. All recipients and donors were genotypically HLA matched except in one. PBSC were harvested on day 5 of G-CSF administration. Follow up ranges from 273-2088 days. *Results.* Median age for BM and PBSC group was 5.4 and 6.2 years. Engraftment was achieved in all cases. Median time to ANC of 0.5×10^9/L in BMT/PBSCT patients was 13 /10 days (range 11-19/9 '15) and for platelets of 20×10^9/L it was 17/14 days (range 14 '28/12'19). aGvHD (grade II-IV) was seen in 30%/26% cases in BMT/PBSCT group. Incidence and severity of chronic GvHD was not statistically different in two groups (BM-24% & PBSC -30%). Six patients rejected the graft: 2 in BM group and 4 in PBSC group. Of the four who rejected the graft from class III, 3 are from PBSC group. DFS in risk classes of the two groups is not significant. Overall survival/disease free survival for the BM and PBSC group as on December 2005 is 73%/65% and 67%/55%. *Conclusion*: The results of PBSCT as a whole or according to risk class remains comparable to BMT. A trend towards less rejection and better disease free survival is seen in class III patients who received BM harvest, but this is not statistically significant.

0824

ALLOGENEIC STEM CELL TRANSPLANTATION 1985-2005. A SINGLE CENTER EXPERIENCE

Y. Floisand,[1] B. Fossum Lland,[2] J. Rislien,[2] G.E. Tjönnfjord,[2] T. Gedde-Dahl d.y,[2] S.A. Evensen,[2] D. Heldal,[2] T. Egeland,[2] B.G. Solheim,[2] D. Albrechtsen,[2] L. Brinch[2]

[1]*Rikshospitalet National Hospital, Oslo, Norway;* [2]*Rikshospitalet University Hospital, Oslo, Norway*

Backgrounds. From 1985 to 2004, 398 adult patients underwent allogeneic stem cell transplantation (ASCT) in our institution. In order to perform a quality control and compare our results with those of other centers, we evaluated the overall survival and the incidence of complications following ASCT. *Design and Methods.* 398 patients received ASCT for hematological malignancies or severe aplastic anemia (SAA); 42.5% female (N=169) and 57.5% (N=229) male. Median age was 39.0 years; during the study period the median age increased significantly (p=0.001). 273 patients (68.8%) received transplants from family donors and 124 (31.2%) from matched unrelated donors (MUD). One patient died during conditioning prior to transplantation. Eligible donors included siblings sharing both HLA haplotypes and haploidentical family donors with a maximum of one antigen mismatch on A or B (serology) or DRB1 on the non-shared haplotype, and MUD identical on A and B (serology), DRB1, DQB1 before 2000 and allelic A, B, C, DRB1, and DQB1 10/10 or 9/10 allelic match from 2000. ASCT was performed due to: Chronic myelogenous leukemia (CML):145 patients (36.4%),128 were transplanted in 1. chronic phase (CP); acute myelogenous leukemia (AML): 131 patients (32.9%), 85 patients had high risk disease as defined by high risk cytogenetics or ≥ 1 complete remission (CR); acute lymphatic leukemia (ALL): 61 patients (15.3%), all patients were categorized as high risk with t(9;22) or relapsed disease; other indications: 61 patients (15.3%). Conditioning regimen for 360 of the patients was oral busulfan 16 mg/kg and iv Cyclophosphamide 60 mg/kg (Bu4Cy2) and for SAA (n=13) cyclophosphamide and ATG. Patients undergoing non-myeloablative transplantation (n=21) were mainly conditioned with fludarabin and TBI (2Gy). Graft versus host disease (GvHD) prophylaxis was cyclosporine A (CyA) and iv methotrexate. Acute GvHD grade II was treated with increased immunosupression (CyA and methylprednisolone (2 mg/kg/day)) and steroid refractory GvHD with ATG or other immunosuppressants. *Results.* Overall survival (OS): The 5 year OS was 58%; 65% and 43% in patients allografted from family donors and MUD respectively. CML: The 5 year OS was 66%; for those with family donor 78% and MUD 46%. AML: The 5 year OS was 56%; for the patients with family donor 61% and MUD 46%. ALL: The 5 year OS was 40%; for patients with family donor 43% and MUD 37%. SAA: The 5y OS was 85%. GVHD: Acute GvHD occurred in 46.7%; aGvHD grade II-IV was 33.2%. The incidence of chronic GvHD was 34.4%; 21.9% limited and 12.6% extensive. *Infections*. Proven or probable invasive fungal infections were registered in 44 patients (12.2%). CMV infection and disease in 74 (18.6%) and 21 (5.3%) patients respectively. *Mortality*. Mortality before day 100 was 11.4% and 25% in the family donor and MUD groups, respectively. Overall non-relapse mortality was 29%. *Conclusions.* Our results compare favorably with previously published series, both single centre studies and multicenter studies.

0825

EXTRACORPOREAL RADIOPHERESIS FOR ACUTE AND CHRONIC GRAFT VERSUS HOST DISEASE

M. Velasquez-Lopera, F. Cuellar-Ambrosi, L.A. Correa, J.C. Wolff, C. Fonseca, M. Rojas, L.F. García

Universidad de Antioquia, Medellin, Colombia

Graft vs host disease (GVHD) is the main immunological complication of hematopoyetic transplants. Unfortunately pharmacological therapies do not always effectively control severe and progressive cases. Extracorporeal photopheresis has been proposed as an effective procedure for GVHD treatment. As an alternative to this treatment, we evaluated the action *Extracorporeal radiopheresis* (*ex vivo* leukocytes irradiation with minimal doses of γ irradiation). Clinical, immunological and skin histological evolution of patients with acute and chronic GVHD who received only pharmacological therapy were compared with

patients who received the pharmacological therapy plus Extracorporeal radiopheresis twice a week. Apoptosis induction, phenotype of dendritic cell subsets and cytokine production were evaluated *in vitro*. *Results.* 15 patients with aGVHD and 15 with cGVHD were studied. In aGVHD grade IV neither treatment affected the long-term survival. Nevertheless, in 3/4 patients (75%) who received radiopheresis, diminution of clinical symptoms (gastrointestinal bleeding and skin rash) was observed since the first week of treatment, improving the quality of life. This effect was also observed at later in patients receiving only pharmacological therapy. In cGVHD, 7 patients received radiopheresis, 5 (71.4%), improved the skin pain since the first week and the skin sclerosis after 6-12 months. In one patient, control of cGVHD progression was obtained. Four of these patients had previously received pharmacological therapy without control of the GVHD. 1/8 patients that received only pharmacological therapy, three improved (37.5%), in three (37.5%), were control of cGVHD progression and two got worse (25%). Histological skin follow-up showed that in aGVHD severity score were one grade lower on all radiopheresis cases evaluated. In patients that received pharmacological therapy only, 80% were the same grade, 20% got worse. For cGVHD skin biopsies were made after 6-12 months. Lower or same histological grade were observed in six patients who received radiopheresis and four patients with only pharmacological therapy, 87,5% and 50% respectively. Induction of apoptosis in cells that received radiopheresis was evaluated with DIOC-6/BE. No changes in the phenotype of dendritic cells differentiated from monocytes with IL-4+GM-CSF, were observed. *Summary*: Better clinical and histological therapeutic effects were observed on patients who received Extracorporeal Radiopheresis. Multicentric studies will contribute to evaluate this therapy in a larger number of patients.

Funding: Supported by COLCIENCIAS (grant 1115-04-12954).

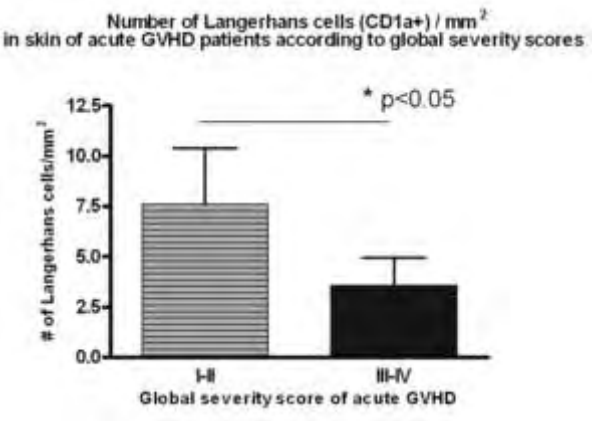
0826

IMMUNOHISTOCHEMICAL EVALUATION OF INFILTRATING CELLS (CD4+, CD8+, AND CD56+) AND LANGERHANS CELLS IN SKIN OF GRAFT VERSUS HOST DISEASE PATIENTS

M. Velasquez-Lopera,[1] A. Vargas,[1] G. Arellano,[1] L.A. Correa,[1] J.C. Wolff,[1] G. Sanclemente,[1] J.C. Villamizar,[1] A. Jaramillo,[2] L.F. García,[1] F. Cuellar-Ambrosi[1]

[1]*Universidad de Antioquia, Medellin, Colombia;* [2]*Rush University Medical Center, Chicago, USA*

Graft vs. host disease (GVHD) occurs in about 60-80% of allogeneic hematopoietic stem cell transplant recipients due to mismatching of major and minor histocompatibility antigens. Cutaneous involvement is the most frequent clinical manifestation of GVHD. In this regard, Langerhans cells (LC) have been shown to play an important role in the pathogenesis of GVHD as antigen-presenting cells through both the direct and indirect allo-recognition pathways. Thus, we carried out an immunohistochemical study to determine the characteristics of the infiltrating T cells (CD4+ and CD8+) and NK cells (CD56+) as well as LC (CD1a+) on skin biopsies of GVHD patients and their correlation with global severity scores. Forty-two patients were allo-transplanted between June, 1998 and December, 2002. Twenty-nine (69%) patients developed GVHD; among these, 15 (36%) developed acute GVHD, 5 (11.9%) developed chronic GVHD, and 9 (21.4%) developed acute and chronic GVHD. Immunohistochemical enumeration of CD1a+, CD4+, CD8+, and CD56+ cells were performed in paraffin-embedded punch skin biopsies taken mainly from the thorax. Among the 24 cases with acute GVHD, skin involvement was observed in 23/24 (95.8%) patients, most of them with G I-II scores. Intestinal GVHD was observed in 20/24 (83.3%) patients with 15 (75%) patients with G I-II scores and 5 patients (25%) with G III-IV scores. Hepatic GVHD was observed in 8 (33.3%) patients, 5 (62.5%) of those patients with G I-II scores. The number of LCs/mm2 in dermis and epidermis was significantly lower in cases with major global severity scores: Normal skin donors: (mean±SD) 15.6±1.6, acute GVHD G-I-II: 7.5 ± 8.8 and G-III-IV: 3.6±2.7 (p<0.05). An increase in the ratio of infiltrating perivascular and epidermal CD8+ T cells was observed and it was inversely proportional to the number of LCs on epidermal and dermal layers of the skin. There was no increase of CD56v NK cells in patients with acute GVHD as compared to normal controls. Figure 1. Extensive chronic GVHD was seen in 7/14 (50%) patients. The number of LC was similar in limited and extensive chronic GVHD (9.5±4.2 and 9±12.7, respectively). In *de novo* chronic GVHD, the number of LCs was higher (13.2±4.6), than in progressive (7.3±0) or quiescent (2.7 ± 3.9) GVHD (p=0.05). Scleroderma-like presentation showed higher number of LC (9.7±6.7) as compared to lichen-like lesions (7.3±0).



No increase in the ratios of infiltrating epidermal and perivascular CD8+, CD4+ or CD56+ cells was observed. In summary, in acute severe systemic GVHD, a significantly lower number of LCs and higher number of CD8+ T cells were observed. These changes were not observed in chronic GVHD. This study indicates that skin CD8+ T cell/LCs ratios could be used as an additional tool for diagnosis and follow-up of GVHD.

Funding: Supported by Universidad de Antioquia.

0827

DYNAMICS OF LINEAGE-SPECIFIC CHIMERISM IN PATIENTS AFTER NON-MYELOABLATIVE HEMATOPOIETIC STEM CELL TRANSPLANTATION

O. Horky, J. Mayer, M. Borsky, J. Pospisilova, M. Krejci, D. Dvorakova

Faculty Hospital Brno, Brno, Czech Republic

Backgrounds. Monitoring of engraftment by assessing chimerism has become routinely used method after allogeneic hematopoietic stem cell transplantation (HSCT). Information about the proportional degree of donor and recipient hematopoiesis and its dynamics in time is particularly important in patients receiving non-myeloablative preparative regimen. *Aims.* The purpose of this study was to describe some aspects of correlation between persistence of lineage-restricted mixed chimerism (MC), complete donor chimerism (CDC), minimal residual disease (MRD), risk of graft versus host disease (GvHD) and relapse. We retrospectively analyzed data of chimerism obtained from 21 patients who underwent non-myeloablative HSCT for chronic myeloid leukemia (CML) in our centre between June 1998 and December 2005 (827 chimerism quantification of whole blood samples, 259 lineage-restricted ones). The conditioning regimen consisted of fludarabine, busulfan and ATG Fresenius. GvHD prophylaxis was cyclosporine A with or without MMF. *Methods.* Assessment of chimerism was carried out by singleplex amplification of variable number of tandem repeat (VNTR) or short tandem repeat (STR) regions - mainly with capillary electrophoresis with fluorescence detection (apart from the beginning when densitometry was involved). Since September 2005 real-time quantitative polymerase chain reaction (RQ-PCR) of insertion/deletion polymorphism was adopted. Detection of MRD was performed by competitive nested PCR and by reverse-transkriptase RQ-PCR. Individual leucocyte subsets (B cells, T cells, NK cells, granulocytes and monocytes) were fluorescence-activated cell sorter (FACS)-sorted according to corresponding antigens. *Results.* Observed patterns are mentioned bellow: 1) MC in whole blood along with MRD negative samples always means that autologous population comes from a lymphoid line. 2) MRD positive samples correlate with appropriate MC in granulocytes. 3) MRD positive samples along with CDC in whole blood are caused by limited sensitivity of VNTR-/STR- PCR (repeatedly retrospectively verified by RQ-PCR, which showed microchimerism). 4) Achievement of CDC is preceded with CDC in T-cells. However CDC in T-cells is not necessarily followed by CDC in whole blood (failure of graft versus leukemia (GvL) effect). 5) MC (even in T-cells) does not always means protection from GvHD. *Conclusions.* Our preliminary data demonstrate that lineage-restricted chimerism analysis allows better understanding of hematopoiesis recovery after HSCT and can significantly contribute to interpretations of relations between chimerism and MRD or GvHD. On the other hand, however, the predictable value of chimerism and especially microchimerism must be further investigated.

0828

ALLOGENEIC BONE MARROW TRANSPLANTATION IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

S. Cantero, J. De la Rubia, M. Blanes, G. Sanz, J. Martínez, J.I. Lorenzo, A. Sempere, N. Puig, L. Algarra, G. Martín, C. Jimenez, M.A. Sanz

Hospital La Fe, Valencia, Spain

Background and Aims. Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired crhonic clonal hematological disorder of pluripotent stem cells. That disease may be complicated by myelodisplastic syndrome and leukemic conversion. Currently, allogeneic bone marrow transplantation (BMT) is the only curative approach. *Patients and Methods.* Between November 1990 and October 1998, six patients (2 F/4 M) with a median age of 26.5 years (range 17-38) underwent BMT for PNH at our institution. BMT was done due to severe progressive cytopenias in five patients and frequent recurrent hemolytic crisis in one. Median time from diagnosis to BMT was 40 months (range 7-58). Five patients were transfusion-dependent and three of them had received several lines of therapy before BMT (steroids, immusupresors and danazol). Four donors were genotypic HLA-identical siblings, one was a non identical sibling donor (major mismatch to one class A HLA antigen) and the remaining one was an unrelated HLA-identical donor. Conditioning regimen consisted of busulphan (16 mg/kg) and cyclophosphamide (120 mg/kg). Graft versus host disease (GvHD) prophylaxis consisted of cyclosporine and a short course of methotrexate. *Results.* The median number of nucleated cells infused was 2.1×10^8/kg (range 1.8-3.9). Time to achieve a granulocyte count > 0.5×10^9/L and a platelet count > 50×10^9/L was 17 and 82 days, respectively. Full donor chimeras was observed in every case although one patient presented mixed chimera 9 years after BMT. In this case, peripheral blood stem cells (PBSC) from the same donor were infused without previous conditioning. Currently, five months after this PBSC infusion the patient is well with complete chimera but with thrombocytopenia. Overall, four patients developed acute GvHD (one grade I and 3 grade III-IV). Chronic GvHD was extensive in four patients. Four patients are alive at +102, +118, +142 and +182 months, and two patients died at 11 and 97 months after BMT because of septic shock. *Conclusions.* Allogeneic BMT is a curative and suitable approach for selected patients with severe PNH. BUCY2 as conditioning regimen was able to eradicate the abnormal PNH clone. GvHD is the complication most frequently observed.

0829

INDACTION INSTEAD OF INDUCTION: NON-INTENSIVE AML/MDS TREATMENT WITH LOW-DOSE DECITABINE PRIOR TO REDUCED-INTENSITY CONDITIONING AND ALLOGENEIC BLOOD STEM CELL TRANSPLANTATION OF OLDER PATIENTS

M. Lübbert, H. Bertz, B. Rüter, R. Mertelsmann, J. Finke

University of Freiburg, Freiburg, Germany

Patients (pts) undergoing allogeneic stem cell transplantation for AML or high-risk MDS usually receive standard induction chemotherapy first, with the goal of achieving a complete remission (CR) or at least best response to conventional chemotherapy. This disease control is often necessary to bridge the time needed for identifying an unrelated donor in case no sibling donor is available, although CR is no absolute prerequisite for successful allografting following RIC (Bertz et al., J Clin Onc 21:1480, 2003). However, prior induction chemotherapy may result in toxicities and prolonged cytopenia, related severe infection like aspergillosis prohibiting or compromizing subsequent allografting. Recently, non-intensive treatment with low-dose azanucleosides has been developed for older pts with high-risk MDS. Low-dose Decitabine (DAC) shows a particularly interesting activity in inducing cytogenetic remissions in MDS pts with poor-risk cytogenetics (38%, *Lübbert et al., Br J Haematol 114:349, 2001*). The role of DAC prior to allografting has not yet been determined. Here we report our single-center experience of 9 consecutive pts treated with low-dose decitabine (DAC) for MDS (n=6) or AML (n=3). The median number of DAC courses given was 3 (range 1-13) with CR and PR as best response in 4 and 1 pt respectively, stable or persistent disease in 3 and 1 pt, resp. Allografting was either done as consolidation at time of best response (n=4) or as salvage for relapsed/refractory AML/MDS (n=5). Median age at time of allografting was 65 years (range 63-71). After conditioning with either FBM (n=7) or Flu/2 Gy (n=2), pts were transplanted from either matched sibling (n=2) or unrelated donor (n=7). One pt died before engraftment on day +13 due to infection. The other 8 pts engrafted, and no unexpected toxicities were noted. 5/8 pts have died either due to relapse (n=3) or infection in CR (n=2). Three pts are alive in CR at the time of this report (+3, +5, +17 months following transplant). Prior to transplantation two of them were in PR and CR following 2 and 8 courses of DAC, respectively, one had beginning relapse of AML after 13 courses. In conclusion, we show that RIC and allografting in older pts with AML/MDS following DAC treatment is feasible, and no unexpected toxicities have occurred. DAC pretreatment can induce improvement or even remission of myeloid neoplasia with negligible nonhematopoietic toxicity, thus bridging the time period of donor search prior to allografting. Since even multiple courses of low-dose DAC are unlikely to be curative without subsequent allografting, the allogeneic option in these pts should be explored more often. Response to DAC and transplant risk-assessment (comorbidities, performance status etc.) will likely be major determinants of optimal timing of allografting in this setting.

0830

ALLOGENEIC STEM CELL TRANSPLANTION IN MULTIPLE MYELOMA

A.M. Carella,[1] M.M. Greco,[2] M. Nobile,[2] P. Musto,[2] L. Savino,[2] M. Centra,[3] G. Di Giorgio,[3] M. Troiano,[4] E. Piazzolla,[4] S. Parisi,[4] N. Cascavilla[2]

[1]*IRCCS CSS Hospital, San Giovanni Rotondo, Italy;* [2]*IRCCS CSS Hospital, San Giovanni Rotondo, Italy;* [3]*Transfusional Center, IRCCS CSS Hosp., San Giovanni Rotondo, Italy;* [4]*Radiotherapy, IRCCS 'CSS' Hospital, San Giovanni Rotondo, Italy*

Backgrounds. HD chemo/radiotherapy followed by autologous stem cell transplantation (SCT) has been associated with improved outcome in MM. Unfortunately, following autologous SCT almost all pts had progressive disease. *Aims.* We evaluated the outcome of 29 pts (17 M, 12 F) with stage III MM treated with hematopoietic SCT. Twenty-three, 6 and 1 pts underwent a matched sibling donor allogeneic transplant after a reduced intensity conditioning regimens (RICT), a matched sibling donor SCT and an unrelated hematopoietic SCT, respectively. Twenty-two pts were treated with autografting followed by reduced intensity conditioning allotransplantation. All these patients received HD Melphalan (200 mg/m^2) followed by autologous PB-SCT. After a median of 90 days, the pts underwent RICT (Fludarabine + 2 Gy TBI). Acute GVhD prophylaxis consisted of MM and cyclosporine. Chimerism analysis was performed using STR-PCR and donor engraftment was evaluated at day +15,+30,+45,+60,+90 on unfractionated BM cells. All pts received a HLA identical donor mobilized PBSC and the graft contained a median of $3{,}3\times10^6$ (range 1-6,8) $^+$ cells/kg body weight. After RICT, on day +15, 3 (13%) pts showed a complete donor chimerism; on day +90, 21 (90%) showed a complete donor chimerism; two pts with mixed chimerism received a DLI on day +30 and one of these achieved full donor chimerism. *Results.* Grade II-III acute GVHD occurred in 4 pts (17%) but no patient died. Five patients (22%) developed a mild and 6 (26%) an extensive chronic GVHD . After RICT 8 pts (35%) achieved CR and they are in CCR at +57,+57,+51,+49,+46,+20,+14 and +15 months; 2 (9%) pts show near CR and 3 (13%) are in PR. Ten pts not in CR showed a progressive disease and six of these died. With a median follow-up of 22 months, 17 (74%) are alive. Six and one patients received a related and unrelated hematopoietic SCT, respectively. The pre-transplant high-dose preparative regimen included CY+TBI. On day 0 all collected PBSCs were infused. GVHD prophylaxis included cyclosporine and short-course methotrexate; ATG was added in the unrelated transplant. All patients showed a complete donor chimerism at the time of engraftment. Grade II-IV acute GVHD occurred in 2 pts and 1 of these died. All pts developed mild chronic GVHD. One patient relapsed and died 24 months after allogeneic SCT. To date, 5 patients are alive and 3 of them are in CCR at + 28 +4 (ALLO) and +21 (MUD) months. *Conclusions.* We demonstrated that survival after allogeneic transplantation is favourable: 74% of all pts achieved CR or PR. The 100-day TRM was low (only 4%) and no patient died after RICT. Pancytopenia after RICT was minimal and sustained allogeneic stem cell engraftment occurred in 95% of patients. A good correlation between GVHD, full chimerism and remission was found. All patients in CR or NCR developed acute/chronic GVHD and the presence of GVHD correlated with a lower relapse rate. In all patients (RICT, ALLO TMO and MUD) the achievement of CR was gradual and a constant regression of the monoclonal band was observed.

0831

TACROLIMUS AND METHOTREXATE FOR THE PROPHYLAXIS OF GRAFT-VERSUS-HOST DISEASE AFTER UNRELATED DONOR CORD BLOOD TRANSPLANTATION FOR ADULT PATIENTS WITH HEMATOLOGIC MALIGNANCIES

T.M. Mori, T. Nakazato, A. Aisa, R. Yamazaki, A. Mihara, Y. Ikeda, S. Okamoto

Keio University School of Medicine, Tokyo, Japan

Backgrounds. Although allogeneic cord blood transplantation (CBT) has been increasingly used as a therapeutic option for hematologic malignancies, the prophylaxis of GVHD has varied significantly among different studies and has included cyclosporine A (CSA) alone or in combination with prednisolone/methylprednisolone, short-term methotrexate, or anti-thymocyte globulin. *Aims.* We present the outcome of CBT for adult patients who received tacrolimus and short-term methotrexate (MTX) for GVHD prophylaxis. *Patients and Methods.* Eighteen patients with hematologic malignancies underwent cord blood transplantation (CBT) from unrelated donors after having been conditioned with myeloablative (n=13) or reduced-intensity (n=5) regimens, and received tacrolimus and methotrexate (15 mg/m^2 on day 1, 10 mg/m^2 on days 3 and 6) as a graft-versus-host disease (GVHD) prophylaxis. The median number of nucleated cells of infused cord blood was 2.66x107/kg of patient body weight. *Results.* Engraftment was achieved in 16 of the 18 patients. The median time to absolute neutrophil count >0.5×10^9/L was 21.5 days (range 17-32), and the median time to platelet count >2.0×10^9/L was 36 days (range 26-57). Of the 16 evaluable patients, 5 and 8 patients had grades I and II acute GVHD, respectively, and none had grades III/IV acute GVHD. The cumulative incidence of grade II acute GVHD was 44.4%. Chronic GHVD occurred in 7 of 15 evaluable patients (limited-type 3, extensive-type 4). Infectious complications were common, including septicemia in 10 patients, CMV disease in 3 patients, and fatal invasive aspergillosis in 1 patient. Of the 18 patients, 14 were alive and disease-free between 173 and 1514 days after CBT (median 746 days), and the probability of disease-free survival at 2 years was 79.1%. *Conclusions.* These results suggested that tacrolimus and short-term methotrexate effectively prevent the occurrence of severe acute GVHD after unrelated CBT, and could contribute to a higher survival rate, although the management of infectious complication is essential.

0832

INCREASING MIXED CHIMERISM DETECTED WITH SHORT TANDEM REPEATS DEFINES A GROUP OF PATIENTS WITH POOR OUTCOME AFTER REDUCEDINTENSITY CONDITIONING ALLOGENEIC PERIPHERAL BLOOD STEM-CELL TRANSPLANTATION WHICH CAN BE IMPROVED BY IMMUNOTHERAPY

M. Alcoceba, A. Balanzategui, P. Martín-Jiménez, M.E. Sarasquete, M.C. Chillón, C. Santamaría, L. Marín, R. García-Sanz, D. Caballero, J.F. San Miguel, M. González

Hospital Universitario de Salamanca, Salamanca, Spain

Introduction: Recent studies indicate that patients with an increasing mixed chimerism after allo-PBSCT have a significantly enhanced risk of relapse. However, as long as we now, none of them have focused on RIC transplantation. *Aim*: To study the relationship between the degree of chimerism and the frequency of relapse, graft rejection, graft-versus-host-disease (GVHD), overall survival (OS) and event free survival (EFS) in patients who have received RIC allo-PBSCT. *Patients*. 102 consecutive stem cell transplantations (SCT) with peripheral blood stem cells from identical MHC sibling donors with reduced intensity conditioning at a single center were included in the study. Their characteristics were: median age 53 (23-69); Male/Female: 64/38; Sex disparity: 50%; Diagnosis: 21 MM, 19 NHL, 17 AML, 14 MDS, 11 CLL, 8 HL, 5 ALL, 4 CML, 2 CMPD, 1 CLL+HL. Two patients died prior to be evaluated, while the remaining cases were valuable for acute GVHD (aGVHD). In addition, 78 patients were included in the analysis for chronic GVHD (cGVHD). 77 were analysed by serial and quantitative chimerism analysis at days +28, +56 and +100. The mean follow-up was 335 days (21-2302). *Methods.* After genomic DNA extraction from bone marrow samples, PowerPlex™ 16 System kit (Promega Corporation, Madison, WI) was used to amplify 16 STR regions (15 plus gender marker, Amilogenin). The amplified products were analysed using GeneScan 2.1 (Applied Biosystems, Foster City, CA) after electrophoresis in the ABIPrism 377 (Applied Biosystems). For statistical analysis, the χ^2 and t-Student tests were used. Log-rank analysis was applied to compare differences between survival curves. Multivariate analysis was carried out according to the cox-regression method. Criteria to define the chimerism status were the previously described by Bader et al (JCO, 2004, 22;1696): Complete chimerism (CC) - No autologous cells at any time after transplantation. Low-level mixed chimerism (LL-MC) - Weak (<5%) autologous signals. Decreasing mixed chimerism (de-MC) - Autologous signals decreasing >5% during follow-up. Increasing mixed chimerism (in-MC) - Autologous signals increasing >5% during follow-up. *Results.* 56/77 revealed CC or LL-MC; in-MC was found in 15 patients and de-MC in 6 patients. Relapse was significantly more frequent in patients with in-MC (12 of 15) than in patients with CC/LL-MC (14/56) or de-MC (2/6; p<0.001). The probability of 5-years EFS was 41% for all patients, with 7% for patients with in-QM and 51% for the rest patients (p<0.001). Within the 15 patients with in-MC, 6 received additional immunotherapy (DLI or bortezomib). This latter group had a significantly higher 5-year OS (67%) than those who did not receive immunotherapy (11%, p<0.05%). Regarding GVHD, patients with CC/LL-MC/de-MC have more incidence of cGVHD (70%) than those with in-MC (15%, p<0.001). *Conclusion*: Serial analysis of chimerism reliably identifies patients at high risk to relapse. Accordingly, patients with increases MC should be actively treated because they are on high risk of relapse.

0833

SERIAL ANALYSIS OF WHOLE BLOOD CELL AND CD3+ T-LYMPHOCYTE CHIMERISM FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION WITH ALEMTUZUMAB CONTAINING REDUCED-INTENSITY-CONDITIONING

S.J. Tauro,[1] R. Lovell,[2] G. Begum,[3] D.J. McMullan,[4] M.J. Griffiths,[4] F.J. Clark,[1] M.A. Cook,[1] P. Mahendra,[1] D.W. Milligan,[2] C.F. Craddock[1]

[1]*University Hospital Birmingham, Birmingham, United Kingdom;* [2]*Birmingham Heartlands Hospital, Birmingham, United Kingdom;* [3]*CR UK Institute for Cancer Studies, Birmingham, United Kingdom;* [4]*West Midlands Regional Genetics Lab., Birmingham, United Kingdom*

Serial analysis of haemopoietic chimerism can be used to predict outcome after allogeneic SCT using a RIC regimen and guide post-transplant intervention. Although alemtuzumab is increasingly used as a component of RIC regimens, there have been few studies of its impact on chimerism status post-transplant. We have therefore measured chimerism following allogeneic SCT in whole blood/marrow nucleated cells (WB) and magnetically selected CD3$^+$ T-lymphocytes in 64 patients with lymphoid (8 high-grade NHL, 18 low-grade NHL, 5 myeloma, 3 mantle cell lymphoma, 9 Hodgkin's lymphoma) or myeloid malignancy (14 AML, 3 MDS, 1 myelofibrosis, 3 CML) conditioned with alemtuzumab and either fludarabine with melphalan (n=46), BEAM (carmustine, etoposide, cytarabine and melphalan) (n=16) or fludarabine/busulphan (n=2). Forty-seven patients received a transplant from an HLA compatible sibling donor and 17 from a matched unrelated donor. All patients achieved neutrophil and platelet engraftment. Donor chimerism was quantified within the first year post-allograft as well as following donor lymphocyte infusions (DLI) by FISH or PCR-based analysis of polymorphic microsatellite regions. 85% of patients demonstrated full donor chimerism (FDC, defined as ≥95% cells of donor origin) in WB within the first 90 days post-transplant. By contrast FDC was only present in the CD3+ compartment of 45% of patients. The proportion of patients with WB FDC declined to 64% by 12 months post transplant whilst the proportion of patients with CD3+ FDC remained constant. Thirteen patients received DLI using escalating CD3+ doses for management of mixed chimerism (MC) including 6 with evidence of disease relapse. Following DLI, 7 patients achieved FDC in WB and CD3$^+$ compartments and 4 failed to switch to FDC. Seven patients developed acute GVHD post-DLI. Acquisition of FDC in the CD3$^+$ compartment within 90 days post-transplant correlated with the presence of acute GVHD (p=0.003). Sixteen patients relapsed of whom 13 exhibited MC in WB or CD3$^+$ compartments. Three patients relapsed despite the presence of FDC in WB and CD3$^+$ cells. There was a trend towards improved disease free survival in patients who achieved FDC in WB within 90 days of transplantation compared to patients with MC (median 30 months v 11 months respectively). These data define a different pattern of WB and CD3+chimerism after alemtuzumab regimens compared with T-replete RIC regimens and confirm a correlation between chimerism status and outcome post-transplant.

0834

CHIMERISM STATUS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

L. Savino, A.M. Carella, M.M. Greco, M. Nobile, N. Cascavilla

IRCCS Casa Sollievo Della Soffererenza, San Giovanni Rotondo, Italy

With the engraftment of allogeneic transplantation, the patient becomes a real chimera, because of the cohabitation in the same person of a genetic patrimony coming from two different people: the patient (pt) and the donor. The periodic control of chimerism is very important for several reasons. It identifies the cellular population's type present after transplantation, it allows the right somministration of immunosuppressive terapy, supporting the reaching of engraftment, and the timely individuation of a possible disease' s relapse. We have investigated the kinetics of engraftement in 133 pts with different malignancies . Seventy nine (median age:47 range 22-62) received reduced conditioning regimens: 22 Flu/Mel, 26 Flu/Cy and 29 Flu/TBI and 54 pts (median age:33 range 10-58) received myeloablative conditioning regimen.We have also evaluated if CD34 cell dose influence engraftment. Due to its high sensitivity, chimerism's valutation is performed using multiplex PCR coamplification of 16 Short Tandem Repeat loci in a single reaction. Donor/recipient cell population ratio was detected by calculing peak area of PCR products for each informative marker. The median number of informative alleles was 6 (range 3-9). We have evaluated the number of patients that have reached the complete chimerism (CDC≥95% donor's cell) at days +15, +30, +90, +180, +270, +360 and so on. In the subgroups of pts that received non myeloablative conditioning regimens the outcame was respectively: 20/79 (25%), 30/79 (38%), 49/79 (62%), 69/79 (87%) 74/79 (94%).We have effectively show that engraftment's kinetics of non myeloablative transplantation is more gradual in time compared to the myeloablative transplantation. In this last one, the engraftment is more rapid and the complete chimerism is already reached on the 30th day. In non myeloablative transplantation, donor engraftment was evaluated at day +15, +30, +90 and so on, in three subgroups of pts that have received different CD34 cell dose:$<2\times10^6$/kg; $>2<8\times10^6$/kg; and $>8\times10^6$/kg. At the day +15 the kinetics of engraftment resulted significantly correleted to dose (p=0.028), while from the day +30 it didn't significantly differ in the three subgroups (p>0.5). Conclutiosions: The valutation of the transplantation' s kinetics of engraftment has shown that in non myeloablative transplantation is normal to have a mixed chimerism with tolerance host versus transplantation and transplantation versus host, for this reason we saggest the importance of the periodic control of chimerism in order to modify the immunosuppressive terapy in favour of the engraftmen and to identify immediately the desease's relapse. The CD34 cell dose has a noticeable effect only in the early kinetics donor chimerism (1 to 15 days).

0835

HEMATOPOIETIC RECOVERY AFTER LOW INTENSITY CONDITIONING TRANSPLANTS AND STANDARD ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)–COMPARATIVE ANALYSIS

G. Kostova,[1] D. Heim,[2] J. Passweg,[2] A. Buser,[2] A. Tichelli,[2] A. Gratwohl[2]

[1]*Clinic for Hematology, Skopje, Macedonia;* [2]*Hematology Department, Univ. Hospital, Basel, Switzerland*

Aim. To compare hematopoietic reconstitution after low intensity conditioning transplants and standard allogeneic hematopoietic stem cell transplantation (HSCT). *Methods.We* retrospectively analyzed the kinetics of cytopenia of 50 consecutive patients treated with HSCT during a 60 day posttransplant period. Twenty four patients were treated with a low intensity regimen (Fludarabine, 2 Gy total body irradiation) and 26 patients with the standard conditioning regimen. Patients who received the low intensity HSCT were analyzed in two groups, patients with engraftment of donor hematopoiesis and those who rejected the graft. *Results*. Patients treated with low intensity conditioning, regardless of its outcome, experienced significantly less severe cytopenia than the patients from the control group. Except for reticulocytes, the development of cytopeniawas significantly slower in these patients, and the duration of severe cytopenia was significantly shorter. However, full neutrophil recovery (absolute neutrophil count $>1.0\times10^9$/L) took longer in patients with low intensity HSCT. *Conclusions*. The kinetics of cytopenia and hematopoietic recovery after lowintensity conditioning HSCT-significantly differ from standard HSCT. There is no difference in the initial hematopoietic recovery between patients with or without engraftment after low intensity conditioning. This indicates that the onset, severity, and duration of the cytopenia are influenced primarily by the intensity of the conditioning and by the immunosuppressive regimen after transplantation. Effects are more pronounced for neutrophils than for platelets and reticulocytes.

0836

ANTITHYMOCYTE GLOBULIN THERAPY FOR STEROID-RESISTANT ACUTE GRAFT VERSUS HOST DISEASE

M. Ballesteros,[1] C. Ferrá,[2] N. Hernández de León,[1] M. Batlle,[2] D. Serrano,[1] R. Carrin,[1] B. Xicoy,[2] P. Balsalobre,[1] J.M. Ribera,[2] J.L. Diez-Martin[1]

[1]*Gregorio Maran Hospital, Madrid, Spain;* [2]*Germans Trias i Pujol Hospital, Badalona, Spain*

Background and Aims. Acute graft-versus-host-disease (aGVHD) is a major cause of mortality after allogeneic stem cell transplantation (ASCT). Initial treatment includes both calcineurin inhibitor therapy and corticosteroids (2 mg/Kg/d). Steroid-resistant aGVHD (SR-aGVHD) is considered when progression after at least 3 days of treatment is observed, lack of response after at least 7 days of therapy, or incomplete response after a treatment course of 14 days. SR-aGVHD usually develops in 30-60% of patients, needing secondary intervention using antithymocyte globulin (ATG) as an option of rescue therapy for RS-aGVHD. We report the experience of two Spanish institutions with this therapy. Patients and Methods. 155 ASCT has been performed in both institutions in the last 9 years. 67 patients (43.2%) developed aGVHD which required first line therapy including corticosteroids. A complete response (CR) was observed in 33 of them and 34 patients (51.8%) developed SR-aGVHD. ATG was administered for rescue therapy in 21 of them. The characteristics of patients are shown at Table 1. *Results.* Our results are presented at Table 2. CMV infection was observed in 12/21 (57.14%) of ATG treated patients. Long term survivors (3/21) developed extensive chronic GVHD. *Conclusions.* Despite initial clinical improvement (12 responses, 6 of them achieved CR), overall survival was poor in our series (3/21) and one year mortality approached 86% for patients with SR-aGVHD. Only 3 patients are long term survivors, all of them with CR after ATG therapy. It appears that the early use of ATG could improve the SR-aGVHD response rate. Nevertheless, mortality was high due to the lack of response or to opportunistic infections. Of note that 7 NM transplants who developed SR-aGVHD responded to ATG therapy (3 CR, 4 PR).

Table 1.

Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Diagnosis	NHL	ALL	CML	ALL	NHL	NHL	ALL	AML	NHL	MM	CML	NHL	AML	HES	CML	AML	BAL	CML	ALL	AML	ALL
Type of transplant	NM MRD	MRD	MRD	MRD	NM MRD	NM MRD	MRD	URD	MRD	MRD	MRD	NM MRD	NM MRD	NM MRD	URD	MRD	URD	MRD	URD	NM MRD	MRD
Steroid																					
Starting day	+25	+12	+16	+26	+29	+21	+13	+14	+20	+14	+48	+35	+69	+57	+68	+29	+10	+17	+7	+25	+17
GVHD grade	III	III	III	II	III	II	II	III	I	III	II	II	IV	III	IV	III	I	III	I	I	I
ATG																					
Starting day	+29	+16	+23	+31	+34	+27	+20	+20	+46	+31	+53	+40	+83	+67	+74	+54	+14	+66	+25	+40	+39
GVHD grade	III	III	II	III	II	II	III	IV	III	IV	IV	II	III	IV	IV	IV	IV	III	IV	II	IV

MRD: matched related donor; URD: unrelated donor; NM: non-myeloabaltive conditioning; HES: hypereosinopholic syndrome; BAL: biphenotypic acute leukemia.

Table 2.

Patients	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Outcome	CR	NR	NR	CR	CR	PR	NR	NR	NR	NR	PR	PR	PR	PR	NR	CR	NR	CR	NR	CR	PR
Alive	+2108			+1385+1222																	
Death		+76 *	+107 °			+324 *	+59 °	+24 °	+56 °	+36 °	+108 #	+78 *	+95 *	+92 °	+86 °	+139 @	+92 °	+394 @	+63 °	+265 ^	+52 §

*CR: complete remission; NR: no remission; PR: partial remission; LPS: lymphoproliferative syndrome; cGVHD: graft versus host disease; TTP: thrombotic thrombocytopenic purpura; *: sepsis; °: aGVHD; #: LPS: @: cGVHD; ^: relapse; §: TTP.*

0837

CYTOMEGALOVIRUS INFECTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION WITH REDUCED-INTENSITY CONDITIONING REGIMEN

E. Elli, M. Parma, F. Rossini, E. Terruzzi, P. Pioltelli, E. Pogliani

Ospedale San Gerardo, Monza, Italy

Background and aim: Cytomegalovirus (CMV) infection remains one of the most important complications for patients (pts) undergone allogeneic stem cell transplantation (allo-SCT). We evaluated incidence and outcome of CMV infection after allo-SCT with reduced-intensity conditioning (RIC) regimen. Methods. 30 consecutive pts (male: female = 17:13) aged from 38 to 67 years (median: 57) were allografted with bone marrow (3) or peripheral blood stem cells (27) from HLA-identical sibling donors from 2000 to 2005. The underlying hematologic malignancies were: acute myeloid leukemia (AML 8), myelodysplastic syndrome (MDS 7), non-Hodgkin lymphoma (NHL 6), multiple myeloma (MM 5), chronic lymphocytic leukemia (CLL 2), idiopathic myelofibrosis (MFI 1) and chronic myeloid leukemia (CML 1). RIC regimens performed were: TT-EDX (17), FLU-TT-EDX (6), TBI 200 cGy (6), and FLU-TBI (1). CMV donor/recipient status was: positive/positive (27), positive/negative (2), negative/positive (1), negative/negative (0). All pts were weekly evaluated with CMV pp65 antigenemia assay for first month; thereafter antigenemia was determined only when a CMV infection was suspected due to clinical or biochemical features. The decision to switch from prophylactic to pre-emptive therapy was made on the basis of two consecutive positivity or the first positivity > 5/200.000 cells. Acyclovir was given as CMV prophylaxis; pre-emptive therapy consisted of ganciclovir, valganciclovir, foscarnet or cidofovir. Results. a positive CMV antigenemia was detected in 10 pts; all of them were seropositive for CMV before alloSCT. 4 pts had AML, 2 NHL, 1 CLL, 1 MM, 2 MDS. The incidence of CMV infection was 10/30 (33,3%). 7 pts presented only one episode of CMV reactivation, 1 patient two episodes and 2 pts three episodes. Median time of first CMV positive antigenemia was 52 days after allo-SCT (range: 22-356), particularly 8 pts had CMV reactivation before 100 days post SCT. Median positive cells at the first appearance of antigenemia was 3/200.000 cells (range 1-14). Overall, we reported 15 episodes of CMV reactivation, 9 before and 6 after 100 days post allo-SCT. Pts who developed late CMV reactivation showed contemporaneously chronic GVHD or disease relapse. Only 8/15 (53%) episodes were treated. Anti-CMV drugs employed had similar effectiveness with a median time of CMV clearance of 15 days. None developed CMV disease nor died for CMV infection. 5/10 pts who suffered CMV infection are still alive; while 5/10 died for disease progression (4) or GVHD (1). 13/20 (65%) pts without CMV infection are still alive. In the same years, 33 pts received a myeloablative allo-SCT from HLA identical donor: among them 6 pts developed a CMV infection for an incidence of 18%. Conclusions. in our group of pts transplanted with RIC allo-SCT, the incidence of CMV reactivation was 33,3%. Pre-emptive therapy was effective and no patient developed CMV disease.

0838

EARLY AND LATE COMPLICATIONS OF FAMILIAL ALLOGENEIC BONE MARROW TRANSPLANTATION. A SINGLE PAEDIATRIC CENTER EXPERIENCE

R. Parasole, G. Menna, G. De Simone, A. Mangione, V. Poggi, M. Ripaldi

Pausilipon Hospital, Naples, Italy

Backgrounds. Improvement in survival rates after bone marrow transplantation (BMT) have sharpened the need for research on adverse events after treatment. These sequelae can have negative effects on quality of life of survivors and when severe are cause of morbidity and delayed mortality. The current study assesses the incidence of early and late effects by a single paediatric institution. *Methods.* Between January 1995 and December 2005, forty-one consecutive familial allogeneic BMT were performed in thirty-eight children (3 patients received a double transplant), with various haematological diseases (14 ALL, 11 AML, 1 CML in CP, 3 FEL, 2 MDS, 3 AAS, 2 NHL, 5 congenital haematological disorders). The median age of children was 9 years (8 months-15 years), 28 males and 10 female. The stem cell source was bone marrow (BM) in 33 cases, peripheral blood (PB) in 6 patients and cord blood (CB) in 2 children. Acute GVHD prophylaxis was performed by cyclosporine added to short-course MTX in immunocompetent patients. Conditioning regimen was diversified based on primary diagnosis. The mean early complications analysed were acute or chronic GVHD, veno-occlusive disease (VOD) and thrombotic microangiopathy (TTP), infections and haemorrhagic cystitis. All patients underwent a careful long-term follow-up for a rapid identification of endocrine dysfunctions, organ-specific sequelae or secondary malignancy. Results. The median dose of nucleated and $CD34^+$ cell infused were $10,8\times10^8$/kg and $5,2\times10^6$/kg in PB-SCT and $4,6\times10^8$/kg and $3,4\times10^6$/kg in BM-SCT, respectively. Median time to neutrophil recovery more than 500/ml was 10 days for PB and 12 for BM while platelets engraftment more than 30.000/ml occurred in 13 days in PB and 21 days in BM (p=0.02); all patients showed a complete attachment. The total incidence of transplant related mortality (TRM) and disease relapse was 7,3% and 17%, respectively. The OS, DFS and EFS of our patients were 76%, 79% and 70%, respectively. The occurrence of grade III-IV acute GVHD and extensive chronic GVHD was of 17% and 7,8%, respectively, that not influenced significantly OS. VOD and TTP were observed in 14,6% and 4,8% of transplants, respectively. Severe infections, CMV reactivation and grade III-IV haemorrhagic cystitis were respectively 12%, 17% and 14,6%. Long-term complications were observed in 60,5% of patients, prevalently endocrine sequelae (47,5%). No data are available on fertility or sexual dysfunctions, for still young age of patients. In a median follow-up of 37 months (range 3-125 months) from transplant, no secondary malignancies were observed. Conclusions. These results suggest that familial allogeneic BMT is an effective therapeutic procedure in most paediatric haematological disease. Nevertheless, it is burdened by high incidence of late effects that often affect negatively the quality of life of survivors. Then, we believe that the patient and family have to be well informed before transplant about all possible complications of these treatment. Duty of clinicians is a careful, prolonged follow-up for a precocious identification and treatment of long-term effects. Future studies should focus on strategies aimed at reducing late sequelae, probably using conditioning regimen at reduced intensity.

Cytokines and growth factors

0839

ERYTHROPOEITIN (EPO) AS AN IMMUNOMODULATORY AGENT: EPO-ASSOCIATED B CELL PROLIFERATION

O.K. Katz,[1] L.L. Lifshitz,[1] B.C.N. Ben-Califa,[1] S. Prutchi-Sagiv[1], G.M. Gassmann,[2] M.M. Mitelman,[1] N.D. Neumann[1]

[1]*Tel-Aviv University, Tel-Aviv, Israel;* [2]*Institute for Veterinary Physiology, Zurich, Switzerland*

Erythropoietin (Epo) is the key hormone regulating erythropoiesis. Recombinant human Epo (rHuEpo) is thus used as a major treatment for various types of anemias. Studies in the past decade have revealed extramedullary sites of Epo production, along with abundance of Epo receptors in various tissues and cell lines, suggesting that this hormone may actually have pleiotropic activities. Our previous studies have implicated Epo as an anti-neoplastic agent in murine multiple myeloma (MM) models (Mittelman et al., PNAS 2001; Katz et al., 2005). The implication that CD8 type T lymphocytes are involved in the anti-neoplastic effects of Epo raised the possibility that Epo has a wide-range of immunomodulatory effects. We thus investigated the effect of Epo on the immune system, focusing on two experimental models. (a) Epo-injected mice as compared to their diluent-injected counterparts (b) transgenic mice constitutively overexpressing Epo (termed tg6) as compared to their age-matched wild-type siblings. In both experimental models we found increased B-cell responses related to Epo effects. Namely, Epo-treated and Epo transgenic mice displayed higher proliferative responses to lipopolysaccharide (LPS) in-vitro, indicating Epo-associated improved B cell functionality. On the other hand, in-vitro stimulation of splenocytes with T cell specific mitogens (e.g. Concavalin A and anti-CD3) elicited less proliferation in Epo-treated and in Epo-overexpressing mice, as compared to their control non-treated and wild type counterparts, respectively. In accordance with these data, FACS analysis of splenocytes from the Epo transgenic mice demonstrated a moderate decrease in CD4-positive T cells and moderate increases in the CD19-positive B cells and in the natural killer cells. These data are supported by an improved Epo-associated response to antigen. Taken together, we propose that Epo acts as an immunomodulator, thus rendering it a potential adjunct agent in the treatment of various diseases.

0840

IL-10 GENE POLYMORPHISM INFLUENCE THE CLINICAL COURSE OF NON-HODGKIN'S LYMPHOMA

J. Mazur,[1] K. Bogunia-Kubik,[2] T. Wröbel,[1] K. Kuliczkowski,[1] A. Lange[2]

[1]*Wroclaw Medical University, Wroclaw, Poland;* [2]*Lower Silesian Centre for Cellular Trans, Wroclaw, Poland*

Backgrounds. Non-Hodgkin's lymphomas (nHL) are heterogeneous group of lymphoproliferative disorders. In the North America and Europe the most frequent nHL are the B-cell lymphomas. Interleukin-10 (IL-10) is an important anti-inflammatory cytokine, mainly produced by Th2 and B lymphocytes. Many studies have shown that IL-10 may be involved in the pathogenesis of lymphoid disorders. Production of many cytokines is related to its gene promoter polymorphism and this polymorphism could be associated with aggressive form of nHL. We have recently demonstrated that the association between the presence of TGF-β1 high producer genotype - TGF-β1 +869 T/C (Leu10Pro) / 915 G/G (Arg25Arg) and TGF-β1 869 T/T (Leu10Leu) and 915 G/G (Arg25Arg) - and the extra nodal manifestation of the non-Hodgkin lymphoma (Cytokine 2006). *Aim:* In this present study IL-10 gene polymorphisms were analysed in 55 NHL patients and 50 controls. *Methods.* IL-10 gene promoter polymorphisms at positions (-1082 A/G, -819 C/T, -592 A/C) were determined by PCR-SSP technique employing commercial primers (One Lambda, Inc. Canoga Park, CA, USA). *Results.* Only a slight prevalence of ACC among patients as compared to controls was observed (32/55 vs. 21/50, p=0.07). Interestingly, this genotype was more frequently detected in patients with more aggressive disease (17/23 vs. 15/32, p=0.04) and in those with 2 or more extra nodal sites of the disease (11/14 vs. 21/41, p=0.07). To assess if IL-10 ACC genotype (associated with lower IL-10 production) constitutes an independent risk factor of more aggressive course of nHL (multivariate logistic regression analysis was performed). IL-10 ACC genotype together with other clinical and biological factors (patient sex and age, stage and aggressiveness of the disease, presence of B symptoms, serum LDH level) was subjected to this multivariate statistical analysis. Multivariate analysis confirmed the role of ACC genotype as a risk factor of more aggressive NHL manifestation (OR=3.57, p=0.05) in addition to the elevated LDH480 level (OR=3.95, p=0.04). Next analysis performed with respect to the number of extra nodal sites showed, although not highly significant ($0.05<p<0.1$), influence of the presence of ACC genotype. *Conclusion*: the low producer genotype of anti-inflammatory Th2 cytokine IL-10 was found to be associated with an unfavorable course of nHL.

0841

EFFECT OF PTH (1-34) ON HOMING OF HEMATOPOIETIC STEM CELLS

D. Svinareva, I.N. Nifontova, J.L. Chertkov, N.J. Drize

National Hematology Research Centre, Moscow, Russian Federation

Background. Osteoblasts are one of the key components of hematopoietic stem cells (HSC) niche within bone marrow. It was shown that Parathyroid hormone (PTH) treatment leads to simultaneous increase in the trabecular osteoblast generation and in the HSC number. PTH is used in pharmacological concentrations for treatment of patients with osteoporosis. Influence of PTH on properties of hematopoietic precursor cells of different stages of maturation is the point in question. *Aims*. The goal of this study was to test the ability of stromal microenvironment of PTH treated mice to maintain successful engraftment of HSC.

Table 1. Homing of CFU-S and CAFC-28 in BM and spleen.

PTH	*Organs*	*CFU-S*	*% seeding CAFC-28*
Control	Bone marrow	43	34
	Spleen	5	7
	Not in hematopoietic organs	52	59
PTH, 10 μkg/kg	Bone marrow	11	18
	Spleen	8	10
	Not in hematopoietic organs	81	72
PTH, 80 μkg/kg	Bone marrow	9	39
	Spleen	6	1
	Not in hematopoietic organs	85	60

Methods. The seeding efficiency (f-24) of short-term (CFU-S) and long-term (CAFC-28) hematopoietic precursor cells to the stromal cells of bone marrow and spleen of lethally irradiated PTH treated recipients was measured 24 hours after intravenous transplantation. Mice were injected i.p. with PTH 80 mkg/kg 5 days/week for 4 weeks. PTH treated and control mice were exposed to 12 Gy total body irradiation and than injected i.v. with 16×10^6 bone marrow cells, previously subjected to two-hour adhesion to plastic. 24 hours later cells from spleen and pooled femur and tibia were harvested. To measure the content of CFU-S homed to the spleen of bone marrow secondary lethally irradiated recipients were injected with either 1/30 of spleen cells or 1/5 of pooled femur and tibia equivalent. CAFC-28 frequency was analyzed in the homed suspension by limiting dilution analysis with standard assay using MS-5 layers. Four dilution steps had been done, with the concentration being halved each time. The first concentration used was 1/24 of total spleen cell amount and 1/4 of pooled femur and tibia cells per well. *Results*. F-24 of short-term HSC in spleen was the same in treated and control mice, but decreased 3-fold in the bone marrow of PTH treated mice. For long-term HSC f-24 in bone marrow was similar in both groups, whereas in spleen it decreased dramatically up to 7-fold. If femur and tibia represent 14% of total pool of murine bone marrow cells (*Colvin, 2004*) it is possible to calculate homing of HSC in PTH treated mice (see table). About 60% of CAFC-28 did not seed on hematopoietic territory, while among the seeded the only (and insignificant) difference seems to be in distribution of these hematopoietic precursors between spleen and bone marrow. PTH treatment had drastically affected homing of CFU-S, judging by significant decrease in f-24 in treated mice. *Summary/Conclusion*. Lowering of CFU-S homing efficiency by PTH might be not safe for hematopoiesis. It seems necessary to scrutinize the effect of PTH on hematopoiesis, especially due to its adaptation for osteoporosis treatment.

0842

SPONTANEOUS TRANSFORMATION OF LYMPH NODE AND BONE MARROW STROMAL CELLS FROM CANCER PATIENTS

L. Kideryova,[1] R. Pytlik,[1] T. Soukup,[2] J. Karbanova,[2] L. Kucerova,[3] S. Micuda,[2] H. Ruckerova,[2] P. Cervinkova,[1] M. Trneny,[1] J. Mokry,[2] P. Klener[1]

[1]1*st Medical Faculty, PRAHA* [2], *Czech Republic;* [2]*Medical Faculty, HRADEC KRALOVE, Czech Republic;* [3]*University Hospital, HRADEC KRALOVE, Czech Republic*

Background. Until recently, human cells were regarded resistant to spontaneous *in vitro* transformation. Last year, two papers in Cancer Research and Cytotherapy reported that diploid mesenchymal stem cells (MSCs) can convert to malignant phenotype in vitro in presence of high concentration of fetal calf serum. AIMS. We have obtained our first transformed stromal cell line in 2003. Since then, we have been studying conditions neccessary for *spontaneous* in vitro transformation and in vitro and in vivo properties of transformed stromal cells. *Methods*. Lymph node stromal cells were obtained from patients undergoing diagnostic or curative surgical procedure for lymph node (4 patients) or epithelial cancer (3 patients). Bone marrow MSCs were obtained from patients undergoing diagnostic or staging bone marrow biopsy. After tissue disaggregation, cells were centrifuged on Ficoll gradient and mononuclear cells were allowed to adhere to tissue culture plastic. Adherent cells were grown in α-MEM with 10% fetal calf serum (FCS) or in α-MEM with 2% FCS supplemented with dexamethason, ascorbic acid, EGF and PDGF-BB. Surface, cytoplasmatic and nuclear antigens were studied by flow cytometry and immunofluorescence. Cytogenetic analysis was performed after standard G-banding. Transformed cells were injected subcutaneously or intraperitoneally into sublethally irradiated NOD/LtSzRag1null mice and tumors were examined histologically. *Results*. We have obtained transformed stromal cells from all seven lymph nodes grown in α-MEM with 10% FCS. Transformation occured very quickly, during initial expansion in one case and from 5th to 10th passage in other cases. Only two transformed cell lines were obtained from more than twenty bone marrow aspirates and in both cases, the transformation occured during 2nd passage. Before transformation, cell cultures did not undergo neither senescent nor crisis phases and normal cells were very quickly overgrown by morphologically abnormal cells with average doubling time of 38 hours. Immunophenotypically, these cells resembled MSCs and were CD90+, CD166+, CD34-, CD45-, cytokeratin- and CD117+. They were also positive for telomerase, grew without contact inhibition and were unable to differentiate into osteoblasts or adipocytes. Transformed cells were hyperdiploid to hypertetraploid (49-115 chromosomes), with nonrandom pattern of chromosomal gains and losses. When administered subcutaneously into immunodeficient animals, these cells produced locally invasive sarcomas and in several cases, visceral metastases were found after intraperitoneal implantation. On the other hand, cells from the same samples grown in MAPC medium with 2% FCS only retained their usual spindle-shaped morphology, contact inhibition, diploid karyotype and ability to differetiate into specialized cells. *Conclusions*. Stromal cells from cancer patients lymph nodes were prone to quick malignant transformation, while mesenchymal stem cells from bone marrow were much more resistant. For transformation, growth medium with 10% FCS was required in both cell types. After transformation, all the cell lines had very similar phenotype, karyotype and clinical behaviour. Whether the easy in vitro transformation is an inherent feature of lymph node stromal cells or reflects the wide-spread genomic instability of cancer patients remains to be established.

Funding: This work was supported by grants IGA 7448/3, MSM 0021620808 and MSM 0021620820.

0843

DOWN REGULATION OF ACTIVIN A BY LYMPHOMA IN THE BONE MARROW: A POSSIBLE MECHANISM OF BONE MARROW INVOLVEMENT

M.Z. Haran,[1] R. Parmawasan,[2] E. Feldberg,[1] M. Fogel,[1] A. Berrebi[1], M. Shtalrid,[1] L. Shvidel,[1] A. Duek,[1] A. Nagler,[3] M. Leiba,[3] D. Zippori[2]

[1]*Kaplan Medical Center, Rehovot, Israel;* [2]*Weizmann Institue of Science, Rehovot, Israel;* [3]*Chaim Sheba Medical Center, Ramat Gan, Israel*

Backgrounds. Increasing evidence points to the crucial role of the interaction of malignant lymphoma cells with their microenvironment that consists of mesenchymal, endothelial and other cells. The tumor stroma may either promote, or alternatively inhibit, tumor dissemination. Our previous investigations indicated that the stromal cytokine activin A induces apoptotic death of myeloma cells due to its antagonism with the growth promoting effect of interleukin-6. Activin A belongs to the transforming growth factor (TGF) β superfamily. It has initially been studied in the reproductive system, but has also been implicated in the regulation of hemopoiesis; it is an erythroid differentiation factor and is expressed within the bone marrow (BM) microenvironment. Activin A functions are tightly regulated by the competitive inhibitor inhibin A and the binding inhibitors, follistatins. We previously showed that abundance of activin A was restrictive for B-cell production in vitro and that within human nasal polyps activin A expression was widespread, but it was absent from foci of B lineage cells. We were therefore interested to find out whether activin A plays a role in the occurrence of BM involvement.

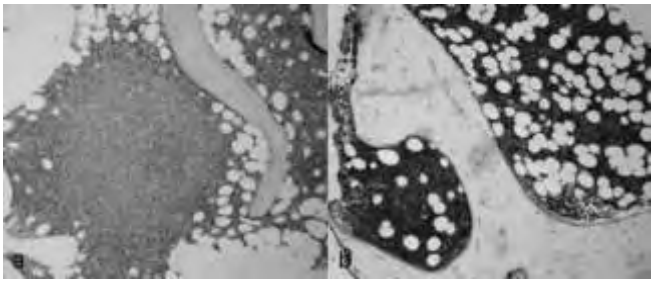


Figure 1. Low power view of activin A in the bone marrow.

Methods. The patient population consisted of all consecutive patients diagnosed with lymphoma between the years 2000-2004. In accordance with the IRB of our hospital, paraffin embedded sections were prepared and immunohistochemical staining was performed using an antibody to activin A. The slides were reviewed by team of 5 investigators and graded separately. We analyzed 17 patients with lymphoma and 3 patients without lymphoma served as controls. Results. Out of 17 lymphoma cases, 10 patients showed BM involvement while 7 patients were without BM involvement. In the former group the level of activin A was significantly decreased in the area surrounding the lymphoid infiltrate (Figure 1A). This was seen uniformly in all the patients except for one, regardless of the original histology of the tumor (follicular or diffuse). The level of activin A in the rest of the BM was similar to the level seen in specimens of reactive BM. In all 7 patients who had no BM involvement we found a diffuse staining for activin A (Figure 1B) (similar to what we saw in patients with reactive BM). *Conclusions.* Lymphoid cells have the ability to migrate to the bone marrow. It is interesting therefore that only some of the patients with malignant lymphomas have BM involvement. This could stem from a difference in the migratory abilities of the lymphoid cells, which is unlikely, or from a difference in their ability to home and flourish in the BM microenvironment. We demonstrated that activin A is significantly down-regulated in the vicinity of the 'metastatic' lymphoma, as opposed to what occurs in normal inflammatory BM. This suggests that an interaction between the lymphoma cell and the BM microenvironment leads to down-regulation of activin A expression and possibly promotes the survival of the lymphoid cells.

0844

EVALUATION OF THE EXPRESSION OF ANGIOGENIC CYTOKINES AND THEIR RECEPTORS IN AUTOIMMUNE MYELOFIBROSIS

R. Rizzi,[1] G. Guaragna,[2] E. Rinaldi,[2] A. Napoli,[3] D. Pastore,[2] G. Specchia,[2] R. Ricco,[3] V. Liso[2]

[1]*Bari University Medical School, Bari, Italy;* [2]*Department of Hematology, Bari, Italy;* [3]*Department of Pathology, Bari, Italy*

Background. Autoimmune myelofibrosis (AM) is an emerging clinicopathological entity, resulting in various degrees of isolated or combined chronic peripheral blood cytopenias. It is defined by a pattern including: increased reticulin fibrosis, not clustered megakaryocytes, reactive lymphoid infiltration in bone marrow biopsies; absence of significant teardrop poikilocytosis and leukoerythroblastosis on peripheral blood smears; normal sized spleen; positive autoimmune serology, possibly fulfilling the classification criteria of an autoimmune disease. It has to be distinguished from different conditions associated with myelofibrosis; among these, the most relevant differential diagnosis is with chronic idiopathic myelofibrosis (CIM), particularly when disclosing autoimmune clinical and/or laboratory features. *Aims*. We purposed to assess the bone marrow stromal changes in AM with particular regard to the

expression of angiogenic cytokines and their receptors, estimation of microvessel density (MVD), and immunophenotype of the lymphoid component. The aim of the present study was to evaluate, by immunohistochemistry, the expression of various isoforms of angiogenic cytokines and their receptors in bone marrow biopsies of AM in comparison with the expression patterns of the same cytokines and their receptors previously detected in CIM and normal bone marrows, as described by Chou et al. (Leuk Res 2003; 27: 499) and Yoon et al. (*Acta Haematol 2000; 104: 151)*, respectively. *Methods*. The tested cytokines and their receptors included platelet derived growth factor (PDGF, PDGFA), basic fibroblast growth factor (bFGF) and its receptors (FGFR1, FGFR2, FGFR3, FGFR4), vessel endothelial growth factor (VEGF) and its receptor (VEGFR1), transforming growth factor β (TGFβ1, TGFβ2, TGFβ3) and its receptors (TGFβR1, TGFβR2). Immunohistochemistry was performed by an immunoperoxidase method with avidin-biotin complex, using specific commercial antibodies (Santa Cruz Biotechnology, USA) on trephine biopsies derived, before treatment, from eight patients (age range: 48-78 years; 6 females) diagnosed as affected by AM. Controls skipping primary antibodies were used as negative controls. *Results*. The immunohistochemical staining for TGF_R1 on endothelial cells of small vessels, and bFGF on megakaryocytes were markedly decreased compared to those observed in CIM samples. For the other tested cytokines and their receptors, AM samples showed patterns of staining intensity and cellular distribution similar to those found in CIM and normal bone marrows. Conclusions. The results of our comparative study suggest that the different bone marrow expression of TGFβR1 in endothelial cells and bFGF in megakaryocytes could be useful to differentiate AM from CIM.

0845

PROINFLAMMATORY CYTOKINES IN THE PATHOGENESIS OF DENGUE FEVER AND HEMORRHAGIC DENGUE FEVER IN VENEZUELA

N. Soyano,[1] A. Müller,[2] A.E. Soyano,[3] C. Cruz,[4] E. Olivo[4]

[1]*Venezuelan Inst. for Scientific Research, Caracas, Venezuela;* [2]*Clinica El Avila, Escuela Luis Razetti, Caracas, Venezuela;* [3]*Escuela Luis Razetti, UCV, Caracas, Venezuela;* [4]*Clinica El Avila, Caracas, Venezuela*

Background. Dengue fever is an flu-like acute viral disease highly prevalent in several regions of Asia and America. It is transmitted by the mosquito Aedes aegypti. In around 30% of the patients, the disease progress towards the haemorrhagic form, which may be potentially life-threatening when haemorrhagic schock develops. The disease has become endemoepidemic in Venezuela, constituting a severe problem of public health. The pathogenesis of the haemorrhagic form of the disease is far from clear, although several cytokines are believed to play an important role. MATERIALS AND METHODS. During the period 2004-2006, especially in the rainy season, numerous cases of dengue fever (DF) were detected in Venezuela. We studied seventy two (72) patients, whose age ranged from 9 to 76 years, were admitted at Clínica El Avila (Caracas, Venezuela) with clinical and laboratory diagnosis of DF. At the time of admission (usually 3-4 days from the beginning of the symptoms), besides the clinical laboratory samples to evaluate routine haematological parameters, coagulation tests (prothrombin time, PTT, thrombin time, fibrinogen and fibrin degradation products, plasminogen and antithrombin), blood chemistry (BUN, creatinine, transaminases), a blood sample was taken to determine plasma concentration of five different cytokines: IL-2 (interleukin-2), IL-6, IL-8 and TNF-α (tumor necrosis factor-α) and GM-CSF. These were quantified with an ELISA assay using a commercial kit (QuantikineTM, R & D Systems, Minneapolis, MN, USA). Twenty (30) apparently healthy blood donors served as normal controls. RESULTS. Of the 72 patients with DF, 24 patients (34%) showed laboratory evidence of haemorrhagic dengue fever (HDF) (platelet count below 100.000/μL and signs of haemoconcentration indicated by an haematocrit greater than 20% of normal value); of these, 11 patients (45.8%) developed petechiae, purpura and severe thrombocytopenia with platelet count below 20.000/μL, requiring administration of plasma components or platelet transfusions. No cases of dengue shock syndrome were observed. Concentrations of TNF-α were found to be significantly increased in 23 patients (31.9%) when compared to normal controls. The increase in TNF-α concentration was positively correlated with the severity of thrombocytopenia. IL-6 was increased only in 11 patients (15.3%), most of them with the severe form of the disease. IL-2, GM-CSF and IL-8 concentrations were not significantly different from those of the normal controls. CONCLUSION. These data suggest that during the development of DF and HDF circulating proinflammatory cytokines such such as TNF-α and IL-6 play an important role in the pathogenesis and severity of the disease. Levels of other cytokines such as IL-2, IL-8 and GM-CSF were unmodified.

0846

EFFECT OF GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) ON DECREASED IL-12P40 PRODUCTION BY CHEMOTHERAPY

T.T. Toubai,[1] J.T. Tanaka,[2] S.O. Ota,[2] T.F. Fukuhara,[3] S.H. Hashino,[2] T.K. Kondo,[2] M.M. Morioka,[4] T.K. Kawamura,[5] N.M. Masauzi,[6] Y.K. Kakinoki,[3] H.K. Kobayashi,[7] Y.K. Kunieda[8], M.K. Kasai[9], H.I. Iwasaki[10], M.A. Asaka,[2] M.I. Imamura[2]

[1]*Hokkaido University, School of Medicine, Sapporo, Japan;* [2]*Hokkaido University Graduate School of M, Sapporo, Japan;* [3]*Asahikawa City Hospital, Asahikawa, Japan;* [4]*Aiiku hospital, Sapporo, Japan;* [5]*Hakodate Chuo Hospital, Hakodate, Japan;* [6]*Hakodate City Hospital, Hakodate, Japan;* [7]*Obihiro Kosei Hospital, Obihiro, Japan;* [8]*Wakkanai City Hospital, Wakkanai, Japan;* [9]*Sapporo Hokuyu Hospital, Sapporo, Japan;* [10]*Sapporo Kosei Hospital, Sapporo, Japan*

Backgrounds. IL-12 is a 70-kDa cytokine comprised of two disulfide-linked proteins (p35 and p40). The highly coordinated expression of p40 and p35 genes to form IL-12 (also called p70) in the same cell type at the same time is essential for the initiation of effective immune response. Granulocyte-colony stimulating factor (G-CSF) affects the balance in the production of anti-inflammatory cytokines. *Aims.* In the present study, we investigated the plasma IL-12 p40 and IL-12 Mix production in patients with B-cell lineage NHL treated with chemotherapy (e.g., CHOP regimen) with or without G-CSF administration. *Methods.* Initially, we examined the plasma IL-12 p40 and IL-12 Mix of the 28 NHL patients before chemotherapy and 8 healthy volunteers. *Results.* We found that plasma IL-12 p40 (191.2pg/ml) and IL-12 Mix (277.4 pg/mL) in patients were higher than healthy volunteers (IL-12 p40:76.4pg/mL, IL-12 Mix:48.5pg/mL) (p=0.04, 0.02). Next, we examined 9 patients with all course of chemotherapy with administration of G-CSF (CG: n=9) and without G-CSF (C:n=9). Serum IL-12 p40 and IL-12 Mix levels with each course were decreased after 10 days chemotherapy, the group of CG were significantly decreased than group C. Serum IL-12 p40 and IL-12 Mix levels in the group of CG (IL-12 p40; mean ± SD, from 142.0 ± 121.8 pg/ml to 24.6 ± 27.3 (10 days), 103.3± 59.0 (17 days) pg/ml, IL-12 Mix; mean ± SD, from 154.2 ± 156.4 pg/ml to 13.3 ± 17.8 (10 days), 97.5± 75.7 (17 days) pg/ml) significantly decreased in comparison with the group of C (IL-12 p40; mean ± SD, from 168.8 ± 77.4 pg/ml to 49.1 ± 43.2 (10 days), 167.8± 67.2 (17 days) pg/ml, IL-12 Mix; mean ± SD, from 222.6 ± 93.3 pg/ml to 48.6 ± 44.1 (10 days), 226.6± 98.2 (17 days) pg/ml) at 10 days and 17 days after chemotherapy (IL-12 p40; p=0.011 (10 days), p=0.006 (17days), IL-12 Mix; p=0.0001 (10 days), p=0.0001 (17 days)). These results showed that administration of G-CSF decreased serum IL-12 p40 and IL-12 Mix levels. Interestingly, plasma IL-12 p40 level in CG group patients with clinical stages and was significantly decreased after chemotherapy than before chemotherapy (mean±SD, -95.6±131.1 pg/mL) (n=16 course) compared with group C (mean±SD, -0.1±35.2 pg/mL) (n=10 course) (p=0.035). However, plasma IL-12 Mix level in CG group patients with clinical stages and was not significantly decreased after chemotherapy than before chemotherapy. Plasma IL-12 p70 levels could not be detected in almost all patients. We analyzed the association with survival rate with administration G-CSF. The overall survival (OS) at 24 months was not significantly differed between both groups(C 58.3% VS GC 80.0%, p=0.67). However, the survival in the patients of clinical stages and with CG group (n=6) significantly improved than C group (n=4) (stages and survival rate 66.6% vs 25.0%, p=0.02). *Conclusions.* We found that chemotherapy with G-CSF decreased IL-12 p40 production. We did not find the difference in overall survival at the present time, however, a longer administration of G-CSF appears to influence on the survival rate by reducing an immunosuppressive IL-12 p40 production.

0847

ANGIOGENIC MOLECULES IN HODGKINS DISEASE: RESULTS FROM SEQUENTIAL SERUM ANALYSIS

F.H. Passam,[1] A. Sfiridaki,[2] G. Tzirakis,[3] J. Moschandrea,[3] P. Roussou,[1] N. Siafakas,[3] M.G. Alexandrakis[3]

[1]*Sotiria Hospital, Medical School Athens, Athens, Greece;* [2]*Venizelion Hospital, Heraklion, Crete, Greece;* [3]*Medical School of Crete, Heraklion, Crete, Greece*

Backgrounds. The induction of new vasculature from pre-existing vessels, termed angiogenesis, is a prerequisite for tumor growth and metastasis and is controlled by a complex network of angiogenic enhancers and inhibitors. Increased angiogenic activity has been demonstrated in

lymphoproliferative diseases including Hodgkin's disease. *Aims.* The aim of the current study was to measure the levels of circulating angiogenic molecules in Hodgkin's patients prior to and after treatment and correlate them to disease stage and prognostic score. Patients-Methods. Serum samples were obtained from sixty patients with newly diagnosed Hodgkin's disease (mean age±SD: 41±19 years) and nineteen healthy individuals (mean age: 39±10 years). Serum samples were obtained from all patients prior to initiation of treatment and in 43 within 6 months of completion of standard ABVD therapy. Six patients relapsed in less than 6 months and 5 died. Two of the 60 patients were diagnosed as Hodgkin's Ann Abor's stage I, 42 stage II, 5 stage III, 10 stage IV. International Prognostic Scores (IPS) of 0, 1, 2, 3, 4 and 5 were recorded for 14, 15, 18, 5, 1 and 3 patients . Elisa measurements were performed using the Quantikine, R & D kits (Minneapolis, MN, USA) for human Hepatocyte growth factor (HGF), Vascular endothelial growth factor (VEGF), Angiogenin, Angiopoietin-2, tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). *Results.* Using the non-parametric Mann-Whitney test, there was strong evidence of higher median concentrations in the pre-treatment group compared to controls for TNF-α (20.8 versus 14.9 pg/ml, $p<0.001$), HGF (1958.8 versus 744.1 pg/ml, $p<0.001$), IL-6 (14.1 versus 3.1 pg/ml, $p<0.0001$) and VEGF (794.4 versus 297.4 pg/ml, $p=0.001$). Angiogenin and angiopoietin-2 levels did not differ from controls. TNF and HGF were found increased in stages III/IV in comparison to stages I/II ($p<0.05$). No statistically significant differences between patients with low and high prognostic score were detected. HGF and VEGF correlated significantly with IL-6 (r=0.56, $p<0.0005$ and r=0.57, $p<0.001$ respectively). HGF, TNF-α, VEGF and angiogenin decreased significantly following effective treatment ($p<0.01$). *Conclusion*. In conclusion, Hodgkin's disease displays an angiogenic activity as depicted by the increased serum levels of a number of angiogenic cytokines. HGF seems to be the prominent molecule in Hodgkin's disease, which may be used to monitor the disease status and the response to treatment.

0848

LEVELS OF CYTOKINES AND OTHER INFLAMMATORY MARKERS IN PATIENTS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

D. Hawrylecka, B. Kusnierz-Cabala, P. Mensah-Glanowska, B. Piatkowska-Jakubas, J. Naskalski, A.B. Skotnicki

Jagiellonian University, Krakow, Poland

Objective: Infectious complications remain a major cause of morbidity and mortality after allogeneic haematopoietic stem cell transplantation (HSCT). Diagnosis and outcome might be improved by using early, sensitive and specific laboratory parameters. The aim of the study was observation of dynamics for some inflammatory markers: IFNγ, TNF- α, interleukin (IL) -18, IL-8, IL-6, serum amyloid A (SAA), C-reactive protein (CRP), procalcitonin (PCT) and neopterin measured at the first 25 days after allogeneic HSCT. *Methods.* We studied 20 patients (mean age 32,4 ± 1,0 years) with haematological malignancies and aplastic anaemia undergoing allogeneic HSCT from related donors with different conditioning regimens and 30 healthy controls. Levels of cytokines and neopterin were measured by ELISA method, CRP and SAA immunonephelmetric but PCT immunoluminometric method. *Results.* Major transplant-related complications (MTCs) included bakteriemia, veno-occlusive disease of the liver, idiopathic pneumonia syndrome, CMV infection, endothelial leakage syndrome and grade > II acute GVHD occurred in 33% of patients. Compared with other complications those with MTCs developed higher levels of IL-18 (896 vs 359,1 pg/ml) and IL-8 (371,8 vs 17,82 pg/ml) beginning from the first day after HSCT procedure); IFN-γ (41,6 vs 15,6 pg/ml at day +6); IL-6 (293,8 vs 64,9 pg/ml at day +8); PCT (2,41 vs 0,86 ng/ml between day +8 up to +12); SAA (663 vs 267,1 mg/l on +6 up to +8); CRP (260 vs 105,7 mg/l on days +8 up to +12); and neopterin (46,3 vs 10,7 nmol/l on day +6 ($p<0,01$). Mutual interrelations confirm correlation between increased concentration of neopterin and IL-6 (r=0,274); with PCT (r=0,546); SAA (r=0,352); IFN-γ (r=0,268); TNF-α (r=0,714); $p<=0,05$. *Conclusions.* IL-18 and IL-8 were shown to be the earliest markers and the best predictors for GVHD after allogeneic HSCT.

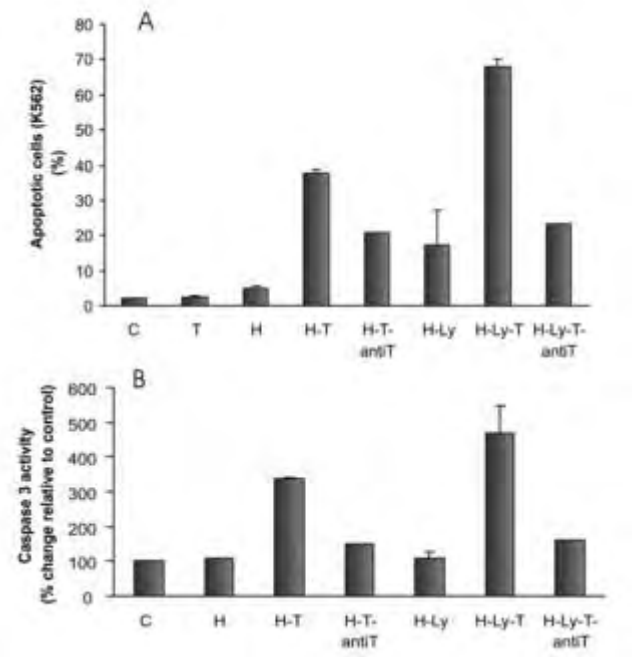
0849

TNF-α INDUCES APOPTOSIS IN CELLS UNDER ERYTHROID DIFFERENTIATION

D. Vittori, G. Pérez, A. Nesse

Universidad de Buenos Aires, Buenos Aires, Argentina

Backgrounds. Inflammatory cytokines inhibit the proliferation of erythroid progenitor cells, among other effects upon iron homeostasis and erythropoietin synthesis, all of which contribute to the pathogenesis of anaemia, a common complication of chronic diseases. Recent studies suggest that the increased release of TNF-α could be responsible for the development of anaemia through the induction of an apoptotic mechanism mediated by death receptors. It has been suggested that this cytokine effect is dependent on the stage of erythroid differentiation. *Aim.* The effect of TNF-α upon differentiation, proliferation and apoptosis was investigated in cells subjected to erythroid differentiation. *Methods.* K562 (erythropoietin-independent) and UT-7 (erythropoietin-dependent) cells were cultured in the presence of haemin (H) for 48 h to study proliferation (Trypan blue test), differentiation (DAF staining) and apoptosis evaluated by apoptotic cells (Hoechst fluorescent nuclear stain) and caspase-3 activity (proteolitic cleavage of chromogenic substrate). mRNA analysis was performed by RT-PCR. *Results.* After 48 h with H, high levels of haemoglobinized cells were observed (K562: 85%, UT-7: 78%). On the other hand, 30 ng/ml TNF-α treatment did not induce significant changes in the development, maturation and viability of both cell lines. Non-differentiated K562 cells were not affected by TNF-α (T) whereas haemin-treated cells were sensitive to the TNF-α proapoptotic effect (Figure A: H-T vs. H, $p<0.002$). This apoptotic action was enhanced by PI3Kinase inhibition with Ly294002 (Fig. A. H-Ly-T vs. H-Ly, $p<0.05$). The negative effects observed in the presence of TNF-α were dramatically decreased by a previous treatment with anti-TNF neutralizing antibody (Figure A). Only in simultaneous experiments with TNF-α and Ly, UT-7 cells cultured in the presence of erythropoietin and induced to differentiation by haemin were induced to apoptosis. This effect resulted significantly higher than that due to signalling inactivation of the growth factor erythropoietin via PI3Kinase (Apoptotic cells: H-Ly-T 92.2±2.4% vs. H-Ly 68.9±5.3%, $p<0.01$). Results of caspase-3 activity measured at 6 h-incubation of cell lysates with chromogenic substrate parallel those of apoptotic K562 cells (Figure B). mRNA levels of Bcl-x, the Bcl-2 related protein that acts as important regulator of cell death, were not modified under the experimental conditions mentioned above. The mRNA of c-FLIP, the suppressor protein of apoptotic signals induced by death receptors, was found diminished in K562 induced to erythroid differentiation but not in UT-7 cells grown under similar conditions. *Conclusions.* During the process of differentiation, cells become sensitive to proapoptotic action of TNF-α. A decrease in c-FLIP expression would explain the apoptosis produced by TNF-α in K562 cells induced to differentiation since this cytokine effect was not observed in differentiated UT-7 cells with non-altered mRNA c-FLIP levels. Besides, cells with different dependence on the growth factor erythropoietin, analysed under similar conditions of erythroid differentiation, show different sensitivity to proinflammatory cytokines. Protective mechanisms against cellular apoptosis caused by TNF-α seem to be mediated by PI3Kinase signalling and proved to be independent from Bcl-x. These findings may have potential implications in the understanding of the mechanisms underlying anaemia in chronic inflammatory diseases.



0850

DEVELOPMENT OF MALIGNANCIES IN MICE TREATED WITH G-CSF FOR A LONG TIME

I.N. Nifontova, D.A. Svinareva, J.L. Chertkov, N.J. Drize, V.G. Savchenko

National Hematology Research Centre, MOSCOW, Russian Federation

Backgrounds. G-CSF is well recognized as a potent mobiliser of hematopoietic stem cells from the bone marrow into the blood, and is being accepted as a regulator of immune responses also. During recent years the use of peripheral blood instead of bone marrow as a source of stem cells has been increasingly employed in the allogeneic transplant setting. Accumulated experience concerning donors treated with G-CSF for stem cells mobilization proved it as a safe procedure. However, the parameters used for the safety evaluation have been rather crude. Few cases discribing several morphological and cytogenetic changes in hematopoietic cells as well as temporal deregulation of some genes in healthy donors were published. Moreover the immunologic complications and leukemia development were noted in G-CSF treated donors. *Aims.* The goal of this investigation was to study the consequences of several courses of G-CSF treatment using low non-mobilising doses on mice model. Methods Female mice (CBAxC57Bl6) F1 and (DBA/2xBalb/c) F1 12-16 weeks old were injected subcutaneously with G-CSF (25 mcg/kg) for 4 days once a month with blood cell count and cytology measured before and after the course, another group of mice of the same strains were injected with G-CSF (5 mcg/kg) for 20 days per month with blood cell count and cytology measured monthly. G-CSF courses have been repeated monthly for half a year. After the termination of treatment blood cell count and cytology were performed in all groups once a month. Total observation time was 21 months. The survival rate was evaluated in experimental and control groups. Results During 20 months of follow up 24 out of 40 G-CSF treated mice died due to unknown cause, 8 mice developed different hematopoietic or other malignancies and disorders and were sacrified. All control mice were healthy with stable number of leukocytes and hemogram. Four-day treatment with 25 mcg/kg/d of G-CSF didn't change the number of leukocytes significantly, while in the group treated for 20 days with a 5 mcg/kg/d the number of leukocytes had slightly increased. Most of the experimental animals had considerable reticulocytosis. Within the first 7 months of follow up among detected disorders myeloproliferative disorders had dominated, afterwards solid tumors were also detected. Most of animals became neutropenic before disease manifestation. All mice injected with G-CSF for 20 days a month developed pus inflammations in site of injection after 3 months of treatment. *Summary/Conclusion.* Long-term treatment of animals with low doses of G-CSF (total 100 mcg/kg per month while mobilizing dose for mice is approximately 1500 mcg/kg) may induce malignant transformation and leads to significant decrease of life-span. Mobilization of hematopoietic stem cells with G-CSF is known to promote deregulation of several genes expression which returnes to normal profile within 2 months. Perhaps prolonged administration of this growth factor leads to dramatic alterations in gene regulation. This probability should be taken into consideration while G-CSF treatment or mobilization of hematopoietic stem cells in healthy volunteer individuals.

0851

PALIFERMIN IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES UNDERGOING AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION (AUTO-HSCT) - PRELIMINARY RESULTS

B. Nasilowska-Adamska,[1] P. Rzepecki,[2] J. Manko,[3] M. Markiewicz,[4] A. Czyz,[5] I. Fedorowicz[6], A. Tomaszewska,[3] B. Piatkowska-Jakubas,[7] A. Wrzesien-Kus,[3] D. Duda[8], K. Halaburda[9], A. Szczepinski[9], K. Warzocha[9], B. Marianska[9]

[1]*Institute of Hematology and Blood Transf., Warsaw, Poland;* [2]*Military Medical Academy, Warsaw, Poland;* [3]*Medical University, Lublin, Poland;* [4]*Silesian Medical Academy, Katowice, Poland;* [5]*K. Marcinkowski University of Medical Sc, Poznan, Poland;* [6]*M.Sklodowska-Curie Memorial Institute, Warsaw, Poland;* [7]*Collegium Medicum of Jagiellonian Univer, Krakow, Poland;* [8]*Lower Silesian Centre for Cellular Trans, Wroclaw, Poland;* [9]*Institute of Hematology and Blood Transf, Warsaw, Poland*

Background and Aims. Oral mucositis (OM) is a frequent complication of myeloablative therapy and HSCT with no effective treatment. In this multi-center study we tested the ability of palifermin (recombinant human keratinocyte growth factor) to reduce the incidence, duration and severity of OM induced by high-dose chemotherapy followed by autoHSCT in patients with hematologic malignancies. We also evaluated the requirement for analgesics and parenteral nutrition administered because of OM, incidence of febrile neutropenia (≥38°C), severe infections and requirement for additional antibiotics. Moreover, the influence of palifermin on the hematopoietic recovery after autoHSCT was assessed in this study. *Methods and Results.* Fifty-six patients with hematologic malignancies were enrolled to the study. Twenty-eight of them (50%) received palifermin (60 microg/kg/day) for three consecutive days before and three consecutive days after conditioning therapy. The median age of the palifermin and control group was 38,3 (range, 19 to 58) and 37,9 (range, 19 to 64), respectively. OM was assessed daily after autoHSCT according to the World Health Organization (WHO) scale. The incidence of OM of WHO grade 1-4 was 42,8 percent in the palifermin group and 96,4 percent in the control group and grade 3-4 was 3,5 percent in the palifermin group and 32,1 percent in the control group. Among all patients the median duration of OM was 2,9 days (range, 0 to 11) in the palifermin group and 9,1 days (range, 0 to 27) in the control group. As compared with control, palifermin was also associated with significant reductions in the use of analgesics (21,4 percent vs. 71,4 percent), opioid analgesics (10,7 percent vs. 53,3 percent) and parenteral nutrition (3,5 percent vs. 28,5 percent). No significant differences in the incidence of febrile neutropenia, severe infections and requirement for additional antibiotics were observed between groups. Also palifermin did not impaired reconstitution of the hematopoietic system (Table 1). The drug was generally well tolerated. Adverse events, mainly rash, pruritus, erythema, generalized oedema, mouth/tongue thickness and discoloration, taste alteration and proteinuria were mild to moderate in severity and were transient. *Conclusions*. Palifermin administration significantly reduced the incidence, severity and duration of OM and did not have negative effect on engrafment in the patients with hematologic malignancies after autoHSCT.

Table 1.

	ANC>0.5×10⁹/L	*PLT>20×10⁹/L*
Palifermin group		
Median (days)	15.8	16.3
range	9 to 42	8 to 45
Control group	15.2	18
Median (days)		
range	8 to 34	8 to 54

0852

EFFECTIVE MOBILIZATION BY PEG-FILGRASTIM PLUS ARA-C CONTAINING REGIMEN IN PRETREATED LYMPHOMAS PATIENTS

V. Pavone,[1] A. Rana,[1] F. Gaudio,[2] C. Del Casale,[3] A. Guarini,[2] R. De Francesco,[1] A. Mele,[1] G. Greco,[1] V. Liso[2]

[1]*Azienda Ospedalira card. Panico, TRICASE (LE), Italy;* [2]*Dipartimento ematologia, universit, BARI, Italy;* [3]*Dipartimento Ematologia, Universit degl, BARI, Italy*

Background. Studies performed on mice and healthy human volunteers have shown that a single dose of pegfilgrastim (Peg-GCSF) is effective in stimulating peripheral blood stem cells (PBSC) mobilization. *Aims.* The aim of this study was to evaluate the efficacy of pegfilgrastim, in combination with salvage chemotherapy, in mobilizing CD34(+) stem cells into the peripheral blood of pretreated lymphoma patients. *Methods* : We studied 27 pretreated patients (Hodgkin's lymphomas=5; non-Hodgkin's lymphomas=22). The median age was 37 years (range 17-60). The patients received a median of 2 previous chemotherapy regimens. Median time from mobilization to harvest was 11.5 days. The efficacy of the mobilizing procedure was tested in lymphoma patients receiving salvage regimen [DHAP (cisplatin 100 mg/m^2 , cytarabine 2000 mg/m^2 × 2) in 17or MAD (cytarabine 2000 mg /m^2× 2× 3 day in 10)] plus pegfilgrastim in 11 or filgrastim in 16. Pegfilgrastim was given as single subcutaneous injection (6 mg) on day +5 post chemotherapy. Filgrastim was given daily (10 µg/Kg) from day +5. Daily monitoring of circulatory CD34+ cells was started from day 8 after the end of chemotherapy.*Results.* Twenty five/27 patients reached the target cell dose of 2,5× 10^6 cells/kg A median of 2 apheresis (range 1-3) was performed. In pegfilgrastim group, a median of 5.27x10^6 CD34$^+$ cells/kg (range1.06-10) was collected. In filgrastim group, a median of 11× 10^6

CD34(+) cells/kg (range 0.09-32.84) was collected. No statistical difference (p=0,06) between the two group (pegfilgrastim vs. filgrastim) was found (Table 1). *Conclusions.* Our results show that pegfilgrastim as an adjunct to HiDARAC based chemotherapy is an effective mobilization regimen in pretreated lymphoma patients also effective as filgrastim based regimen. This approach is to be confirmed in larger series of patients and probably with increased dose of pegfilgrastim and could open new opportunities in stem cell mobilization for poor or non-mobilizers patients with malignant lymphomas.

Table 1. Pegfilgrastim vs Filgrastim in 27 pts (51 apheretic procedures).

	Pegfilgrastim	*Filgrastim*	
N° apheretic procedures	2,1	1,8	
Median day to 1st harvest	13,5 (+11-16)	12,8 (+10-15)	
Median CD 34×106(overall 8,79)	4,96	10,5	n.s.
Poor mobilizer (< 2,5 CD 34×10^6) overall 6 pts (22%)	4	2	n.s.
Very poor mobilizer (< 1 CD 34×10^6) overall 3 pts (11%)	1	2	

(>2,5 CD 34×10^6) overall 21 pts (78%).

0853

LOW DOSE LENOGRASTIM IS AS EFFECTIVE AS STANDARD DOSE IN SHORTENING NEUTROPHIL ENGRAFTMENT TIME FOLLOWING MYELOABLATIVE CHEMOTHERAPY AND PERIPHERAL BLOOD PROGENITOR CELL RESCUE

D.F.L. Nolan,[1] P.W. Johnson,[2] S. Chilton,[2] P. Lorigan,[3] R. Else,[3] P. Smith,[4] J.W. Sweetenham[5]

[1]*Southampton General Hospital, Southampton, United Kingdom;* [2]*Cancer Research UK Clinical Centre, Southampton, United Kingdom;* [3]*Weston Park Hospital, Sheffield, United Kingdom;* [4]*CR UK/UCL Lymphoma Trials Office, London, United Kingdom;* [5]*Cleveland Clinic Foundation, Cleveland, USA*

Backgrounds. G-CSF is widely used following HDT and PBPCR to reduce neutrophil engraftment time. The dose and duration required to gain maximum clinical and economic benefit has not been fully investigated. *Aims.* This double blind placebo-controlled randomised trial was performed to determine whether short course low-dose or standard-dose L would influence recovery of haematopoiesis following HDT and PBPCR. *Methods.* 61 patients (pts) with non-Hodgkin lymphoma (40) or Hodgkin lymphoma (21) undergoing HDT were randomised between May 1999 and November 2004. Pts had normal peripheral blood counts prior to HDT (Hb ≥100g/L, total white cell count ≥3.0×10^9/L, neutrophils (N) ≥1.0×10^9/L and platelets ≥50×10^9/L), and had a minimum 2.5×10^6/L CD34$^+$ cells/kg PBPC previously collected following mobilisation with Cyclophosphamide 3 g/m^2 and G-CSF. All received HDT with BCNU 300 mg/m^2 d-7, Etoposide 200 mg/m^2 od d-5-d-2, Cytosine arabinoside 200 mg/m^2 bd d-5-d-2 and Melphalan 140 mg/m^2 d-1 before return of PBPC on d0. Pts were allocated standard dose L 263 µg daily (20 pts), low dose L 105 mc^3 daily (21 pts) or placebo injections (20 pts). These commenced on day +5 following PBPCR and continued until N≥0.5×10^9/L. Pts received standard supportive care including prophylactic Fluconazole and Acyclovir, but not routine antibacterial prophylaxis, until haemopoietic recovery. *Results.* L at any dose resulted in a significantly shorter median time to N recovery ≥0.1 (10.0 vs 11.0 days, p=0.02) and ≥0.5 (11.0 vs 14.0 days, p=0.0003) compared to placebo. The only significant difference between standard- and low-dose L was in hospital stay (21.0 vs 22.0 days, p=0.04), however L at any dose showed a significant reduction over placebo (22.0 vs 23.0 days, p=0.01). There was no significant difference in blood product support or antibiotic usage between the groups. At a median follow up of 40 months there were 27 confirmed lymphoma relapses and 26 deaths (21 relapsed lymphoma, 1 secondary AML, 4 other). *Conclusions.* Short course low dose L is as effective as standard dose in reducing neutrophil engraftment time following HDT and PBSCR. L at any dose reduces hospital stay when compared to placebo. This approach should be considered for those patients in whom growth factor support is indicated.

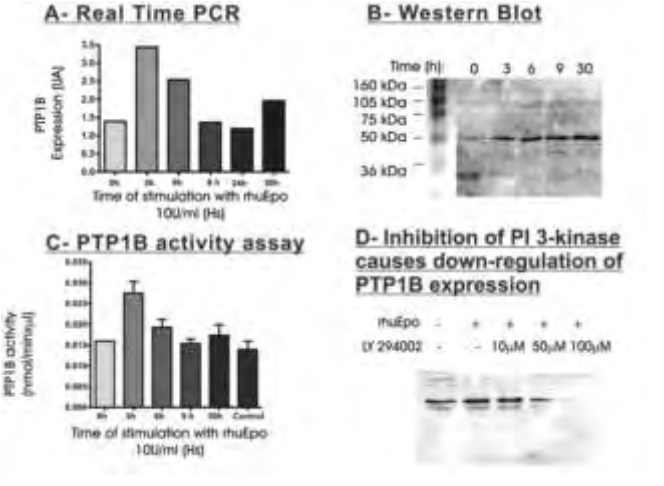
0854

MODULATION OF PROTEIN TYROSINE PHOSPHATASE 1B BY ERYTHROPOIETIN IN UT-7 CELL LINE

M. Callero, G. Pérez, D. Vittori, A. Nesse

Buenos Aires University, Buenos Aires, Argentina

Backgrounds. The central role played by tyrosine phosphorylation of erythropoietin receptor (EpoR) in cell activation by erythropoietin (Epo) has focused attention on protein tyrosine phosphatases (PTPs) as candidates implicated in the pathogenesis of the resistance to therapy with human recombinant Epo. The prototypic member of the PTP family is PTP1B, a widely expressed non-receptor PTP located both in cytosol and intracellular membranes via its hydrophobic C-terminal targeting sequence. PTP1B has been implicated in the regulation of a number of signaling pathways, in particular, those involving tyrosine phosphorylation induced by growth factors, cytokines and hormones such as the down-regulation of EpoR and insulin receptor. Binding of ligand to cell-surface EpoR results in the activation of JAK2 and phosphorylation of tyrosine residues in the cytosolic domain of the receptor. Termination of the EpoR signaling is attributed to the cytosolic SH-PTP1. However, it has been demonstrated that PTP1B also participates in down-regulation of the ligand-activated cell surface EpoR. *Aim.* To investigate the effect of Epo on PTP1B expression. *Methods.* The UT-7 human cell line was used as an Epo-dependent model. Epo was added to serum- and Epo-deprived cells for previous 18 h. After different periods of Epo incubation, cells were lysed and total proteins and RNA were obtained. cDNA was prepared from different RNA samples and PTP1B mRNA level was analysed by Real Time PCR. Total proteins or immunoprecipitates with anti-PTP1B were subjected to Western Blot using anti-PTP1B or anti-PTyr. Immunoprecipitates were also subjected to a PTP1B activity assay with pNPP. Then, the experiment was repeated including pretreatment with LY294002 (PI3K inhibitor) before Epo stimulation. *Results.* An increased and maximun level of PTP1B mRNA was already observed at 3 h of Epo stimulation (figure a). This increment correlates with the induction of PTP1B expression observed by Western Blot (figure b). However, after 9h with Epo, mRNA level returned to baseline while protein expression remained constant. PTP1B Tyr phosphorylation was detectable after 5 min of Epo stimulation and declined within 6 h PTP1B activity increased after 3 h of Epo incubation and diminished to the basal level within 6 h (Figure C). Figure d shows that the pretreatment of UT-7 cells with LY294002 downregulated PTP1B expresion in a dose-dependent manner reaching the highest inhibition at 100 mM LY concentration. *Conclusions.* We have found an Epo-induced expression of PTP1B, associated with increased PTP1B Tyr phosphorylation, suggesting that besides modulating Epo/EpoR signaling, PTP1B suffers a feedback regulation by Epo.



0855

PLASMA CYTOKINE PROFILE IN HEALTHY INFANTS IS NOT INFLUENCED BY VACCINATION

J.L.A.J. Rummens,[1] M. Raes,[1] H. Jongen,[1] V. Peeters,[1] P. Scholtens,[2] B. Maes,[1] K. Hensen[1]

[1]*Virga Jesse Hospital, HASSELT, Belgium;* [2]*Numico Research, Clinical Trials, WAGENINGEN, Netherlands*

Backgrounds. The immune system is a complex mechanism in which cytokines play a critical role in coordinating immunological responses and disease pathogenesis. Deficiencies or imbalances in T-cell cytokine responses at early age may lead to the development of atopic allergic diseases and autoimmune disorders. Additionally, several studies have shown that increased plasma levels of inflammatory cytokines correlate with neonatal sepsis and bacteraemia in infants. Besides, measuring cytokine levels could be a useful tool for the detection of leukaemia-reactive T-cells and for monitoring the immunosuppressive effect of cytotoxic drugs used for the treatment of childhood acute leukaemias. *Aims.* Although plasma cytokine profiles could be very valuable for the clinical-therapeutic monitoring of immunological status in various childhood diseases, rather scarce data are available about normal plasma cytokine profiles in the first years of life. Furthermore, while vaccination is an artificial way to introduce protective immunization, no data are available on the potential impact of vaccination on the plasma cytokine profile. The aim of this study was to profile plasma cytokine levels in normal infants without any infections and to evaluate the impact of vaccination on these parameters. *Methods.* Th1/Th2 plasma cytokine levels (interleukin (IL)-2, IL-4, IL-5, IL-10, tumour necrosis factor (TNF) a and interferon (IFN)-γ) in 164 healthy infants were assessed at two time points, 8 weeks (T8) and 26 weeks after birth (T26). All children were vaccinated with at least 5 different antigens namely DtaP-IPV (Tetravac°, Aventis Pasteur MSD) and simultaneous administration of Hib (ActHIB°, Aventis Pasteur MSD) at 2, 3 and 5 months of age. Cytokines were quantified using the BD cytometric bead array (CBA) kit because it allows the simultaneous measurement of multiple cytokines from small sample volumes. Simultaneously, lymphocyte subsets and classical laboratory parameters like leucocyte count, CRP and immunoglobulins were determined. Group comparisons were compared with the Mann-Whitney U test (nonpaired Wilcoxon test). *Results.* Our results showed no statistically significant difference in cytokine levels between T8 and T26 ($p>0.05$), except for IL-4 ($p=0.015$). In line with these results, B-lymphocytes ($p<0.0001$) T-lymphocytes ($p<0.0001$) and IgE plasma levels ($p<0.0001$) were elevated, reflecting a normal developing immune system. *Conclusions.* As far as we know, this is the first report describing plasma cytokine levels and the potential impact of vaccination on such a large number of healthy infants. For that reason, our values might be very useful in studies on the normal ontogeny of immune cells during infancy. Furthermore, our data can be utilized as age matched references values of cytokine production, which are extremely important for correct interpretations in clinical-therapeutic monitoring of infants during various childhood diseases.

0856

PRO-INFLAMMATORY CYTOKINES AND ATHEROSCLEROSIS IN CHILDREN

N. Lefkou, E. Ioannidou, V. Garipidou, S. Vakalopoulou, V. Perifanis, K. Tziomalos, M. Athanasiou, I. Tsiouris, I. Klonizakis

Ippokrateion University Hospital, THESSALONIKI, Greece

Introduction: Atherosclerosis is a progressive inflammatory disease that initiates in childhood. Family history of premature coronary artery disease is an independent risk factor of atherosclerosis. *Aim*: The aim of this study was to investigate whether the onset of the inflammatory process of atherosclerosis initiates early in children with positive family history of premature coronary artery disease. *Methods.* We study 55 healthy children (5-15 years), 30 (16 male) with positive family history of premature atherosclerosis and 25 children (12 male) without positive family history. We performed macrophages cultures and we measured in the cell culture supernatants the pro-inflammatory cytokines 9L-1a, IL-6 and TNF-α. *Results.* There was a statistically significant differences in the secretion of IL-1α, Il-6, TNF-α measured in cell culture supernatants in children with positive family history. The Il-1α and IL-6 values was related proportionally with the triglycerides values. The TNF-α value was proportionally related with total cholesterol, atheromatous index and triglycerides. Conclussion: Children with positive family history of premature atherosclerosis have highter values of IL-1α, IL-6 and TNF-α secreted in macrophage cultures, in relation with children without a positive history. These observation suggest that any therapeutic intervention targeting in modify that inflammatory index in childhood maybe can lead to a delay of atherosclerosis in the adulthood.

0857

MONOCYTE CHEMOATTRACTANT PROTEIN-1 AND INTERLEUKIN-8 LEVELS IN ACUTE INFLAMMATION INDUCED BY PROLONGED BRISK EXERCISE: CLINICAL IMPLICATIONS

I. Papassotiriou,[1] M. Tsironi,[2] K. Skenderi,[3] G. Chrousos[4]

[1]*Aghia Sophia Children's Hospital, Athens, Greece;* [2]*Sparta General Hospital, Sparta, Greece;* [3]*'Harokopio' University, Athens, Greece;* [4]*Athens University Medical School, Athens, Greece*

Monocyte chemoattractant protein-1 (MCP-1), also known as CCL2, a chemokine that regulates migration and infiltration by monocytes/macrophages, belongs to the CC chemokine subfamily. Interleukin-8 (IL-8), also known as CXCL8, a proinflammatory chemokine with angiogenesis-promoting properties belongs to the CXC chemokine superfamily. Both MCP-1 and IL-8 have important roles in the pathogenesis of many chronic inflammatory disorders, including atherosclerosis and obesity. Their proinflammatory effects are mediated mainly by the CC chemokine receptor 2 (CCR2) and CXCR1/R2, respectively. MCP-1 and IL-8 cause chronic vascular inflammation and induce thrombosis, proliferation and migration of vascular smooth muscle cells, angiogenesis, and oxidative stress. Previous studies indicate that: 1) MCP-1 production from endothelial cells, smooth muscle cells, and regional leukocytes increases in the presence of endothelial dysfunction and atherosclerosis risk factors; 2) MCP-1 and IL-8 expression is increased in atherosclerotic lesions and injured arteries; and 3) eliminating MCP-1 function decreases neointimal hyperplasia after injury and atheroma formation in mice. We studied the association between MCP-1 and IL-8 levels and the degree of inflammation in 15 athletes that participated in the ultra-distance foot race of the 246 Km 'Sparthathlon'. This race consists of continuous, prolonged, brisk exercise. We reported earlier that Interleukin-6 (IL-6), C-reactive protein (CRP), Serum amyloid A protein (SAA) and free plasma DNA levels markedly increased (by 8000-, 152-108- and 10-fold, respectively) over the baseline at the end of the race1. However, IL-6 levels returned to normal by 48h, while CRP, SAA and free plasma DNA remained elevated. Circulated levels of MCP-1 and IL-8 were measured by means of a multi-analyte Biochip Array Technology, using the Evidence analyzer (Randox Laboratories, UK). The measurements were performed before (phase I), at the end (phase II) and 48h post-race (phase III). MCP-1 levels at phase I (216.9 ± 48.5 ng/L) (mean±SE), increased significantly at phase II (592.9 ± 115.7 ng/L) and subsequently decreased at phase III (278.1 ± 62.7 ng/L). At the same time period, IL-8 followed a similar pattern (phase I: 9.4 ± 4.5 ng/L, phase II: 28.5 ± 8.8 and phase III: 8.9 ± 4.3 ng/L). A significant positive correlation between MCP-1 and IL-8 was found at phase III ($r = 0.845$, $p< 0.01$), while this correlation was absent in the other two phases, indicating an independent response of each chemokine to inflammatory stimuli in each athlete. In conclusion, prolonged exercise induces an inflammatory response that is expressed by an increase in circulating MCP-1 and IL-8 levels. Whether these changes have long-term negative effects on the vasculature remains unknown.

References

1. Clin Endocrinol Metab 2005;90:3914-8.

0858

EVALUATION OF THE EFFECTS OF THE CD33-TARGETED DRUG GEMTUZUMAB OZOGAMICIN ON GROWTH AND HISTAMINE RELEASE IN HUMAN MAST CELLS AND BASOPHILS

M.T. Krauth,[1] A. Böhm,[1] H. Agis,[1] K. Sonneck,[1] S. Florian,[1] K. Sotlar,[2] P. Valent[1]

[1]*Medical University of Vienna, Vienna, Austria;* [2]*University of Tbingen, Tbingen, Germany*

Objectives. Mylotarg (gemtuzumab-ozogamicin=GO) has recently been introduced as a novel CD33-targeting drug in clinical hematology. However, despite clinical efficacy in acute myeloid leukemia, GO produces significant side effects including an infusion-syndrome. We have recently shown that mast cells (MC) and basophils (BA) express CD33. In the present study, we investigated the effects of GO on mediator secretion and growth of MC and BA. Methods. Growth-inhibitory effects of GO on neoplastic MC (HMC-1) and BA (KU812) as well as cord blood-derived MC- and BA progenitor cells were determined by counting cell numbers and the numbers of apoptotic cells. The amount of histamine secreted from primary MC and BA were measured after incubation of cells with GO alone or GO together with an anti-IgE antibody. *Results*. MC and BA as well as HMC-1 cells and KU812 cells were found to express CD33 mRNA and the CD33 protein. GO was found to inhibit the growth of HMC-1 cells and KU812 cells as well as SCF-dependent differentiation of MC and IL-3-induced growth of BA from

their cord blood-derived progenitors. The GO-induced inhibition of growth of neoplastic cells was found to be associated with induction of apoptosis. GO neither induced secretion of histamine from MC or BA nor did GO upregulate the anti-IgE-induced release of histamine in these cells. Conclusions. GO counteracts cell growth in normal and neoplastic human MC and BA without inducing release of histamine. Therefore, GO may be considered as a new targeted drug for the treatment of high-grade MC- and BA neoplasms.

0859

ALTERATIONS IN GENE EXPRESSION IN MURINE LEUKEMIA CELLS DEVELOPED AFTER G-CSF TREATMENT

A. Bigildeev, L . Grishchuk, A. Bigildeev, N. Nifontova, A. Svinareva, J. Drize, G. Savchenko

National Hematology Research Centre, Moskow, Russian Federation

Background. Four-day treatment of mice with low (25mcg/kg) G-CSF doses known to be insufficient for mobilization of hematopoietic stem cells into peripheral blood almost halves the content of bone marrow primitive hematopoietic stem cells and doesn't affect the CFU-S number. Female mice (CBAxC57Bl6) F1 12-16 weeks old were subjected to such a course once a month. MPD-like myeloid leukemia with histiocytic sarcoma occurred in one case after 3rd course of G-CSF treatment. Liver tissue was totally substituted by undifferentiated cells with no morphologically definable features. The liver was about 4-5-fold enlarged by sight. Bone marrow and liver cells of the mouse were fully transplantable, recipients became moribund within 17-32 days since cells injection. All ill animals had enlarged liver (M 3,4 ± 0,5 g versus normal 1,38 ± 0,3 g). The developed leukemia was not of virus origin, which was proved by three independent methods. *Aims*. To understand molecular regulation of malignization, differentially expressed genes of interest must be identified, cloned and studied in detail. *Methods*. Subtracted cDNA library from bone marrow cells of normal and leukemic mice was prepared by Suppression Subtractive Hybridization (SSH) method. Several up-regulated genes were further studied by RT-PCR in bone marrow and liver of leukemic mice. Normalization factor was evaluated by 3 housekeeping genes (HPRT1, RPL13A, UBC) by Genorm software. *Results*. The clinically ill mice showed a moderate extent of anemia and reticulocytosis, which was supported by suppressed b-globin expression (top 5 down-regulated genes turned out to be b-globin genes). The expression level of c-abl and G-CSF doubled in bone marrow of leukemic mice compared with the normal bone marrow, while the concentration of CFU-C per 105 cells increased 4-fold (247 ± 31,2 in ill mice versus 57,9 ± 27,0 in control animals, $p < 0.01$). The expression level of genes regulating cell proliferation did not change dramatically - only C-Myc expression increased 3-fold, however concentration of early hematopoietic precursor cells (LTC-IC) decreased about 5-fold (0,75 versus 3,32 per 105 cells in healthy mice). The pronounced changes were revealed in expression of MPO gene (3,4-fold increase). The liver of ill mice consisted of undifferentiated cells. As CD45 expression increased up to 11-fold simultaneously with constitution of liver parenchyma by tumor cell, one may suggest hematopoietic origin of invading cells. CFU-C were also revealed in affected liver (52,5 ± 7,7 per 105 cells).There were minor changes in G-CSF expression in liver cells of leukemic mice, whereas expression of G-CSF-R increased 18-fold compared with normal liver cells. Expression of c-abl also increased. Expression of anti-apoptotic genes was elevated up to 4-fold for bcl-2 and 2-fold for cIAP2. Unlike in the bone marrow, expression of JunB in the liver increased 5-fold. *Summary*. The G-CSF treatment may lead to development of myeloid leukemia with dramatically changed gene expression and high ability to invade liver tissue.

0860

A SINGLE FIXED DOSE INJECTION OF PEGFILGRASTIM TO MOBILISE AUTOLOGOUS STEM CELLS OF EXTENSIVELY PRE-TREATED LYMPHOMA AND MYELOMA PATIENTS; NOT ALWAYS SUCCESSFUL

P.F. Ypma, A.A. Muradin, P.W. Wijermans

HAGAziekenhuis, Leyenburg, THE HAGUE, Netherlands

Backgrounds. In patients with multiple myeloma and refractory or relapsed lymphoma consolidation high-dose chemotherapy combined with stem cell rescue is an established therapy in chemosensitive disease. A commonly used approach to mobilise CD34 positive hematopoietic stem cells into the blood is the administration of granulocyte colony-stimulating factor following a course of chemotherapy. Pegfilgrastim, the pegylated form of filgrastim, is subject to a distinct method of clearance by neutrophilic leukocytes compared to filgrastim. Pegfilgrastim showed in earlier series of patients to be effective in mobilising blood progenitor cells in single fixed doses of 6 mg. as well as 12 mg. The optimal dose and scheduling of the injection and apheresis is not completely established in various patients groups with diverse extents of chemotherapeutic and radiotherapeutic pre-treatment. *Aims.* The primary aim was to study the feasibility of a single low dose of pegfilgrastim in extensively pre-treated patients. *Methods*. Forty-six Consecutive patients with myeloma or relapsed/refractory lymphoma who underwent stemcell mobilisation by identical apheresis techniques using either filgrastim or pegfilgrastim were retrospectively studied. Patient- and disease characteristics, pre-treatment data and mobilising chemotherapy type as well as cytokine dose, apheresis results and neutrophil recovery data were compared. *Results*. Stem cell harvest was performed in 24 patients after administration of pegfilgrastim once (6 mgs.) and in 22 patients after filgrastim. The filgrastim was administered in a median total dose of 4,2 mgs. (7,7 mgr./kg/day) and 11 injections were needed. The apheresis took place after 13 and 13,5 days respectively, with a maximum CD34-count of 8×10^7/L (pegfilgrastim group) and $11,6\times10^7$/L (filgrastim group). Of 24 patients who received pegfilgrastim, 5 patients showed a failure mobilising stemcells (21%). In 2 of those 5 patients harvesting succeeded eventually after additional stimulation with filgrastim and another 2 patients were mobilised in a later stage after an additional course of chemotherapy using filgrastim in high dosage. None of the filgrastim mobilisation procedures failed. A median of $7,2\times10^6$/kg $CD34^+$cells was obtained in 19 patients after pegfilgrastim administration. 6 Patients needed a second- and 1 patient needed a third apheresis procedure to collect enough stem cells (threshold 3×10^6/kg and 6×10^6/kg in myeloma patients). In the filgrastim treated group more $CD34^+$ cells were obtained, 11×10^6/kg, in fewer procedures. The collected number of $CD34^+$ cells per kg bodyweight per ml. of processed volume during the apheresis procedure was higher in the filgrastim group, 382×10^6/kg ml (pegfilgrastim) vs. 803×10^6/kg ml (filgrastim). *Conclusions*. A fixed dose of pegfilgrastim (6 mg) is not sufficient to achieve adequate stemcell mobilisation in all patients. Failure was observed in 21% of the patients. The number of $CD34^+$ cells collected and the efficiency of the apheresis procedure appear to be higher in the patient group treated with filgrastim.

0861

CIRCULATING VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) AND ITS SOLUBLE RECEPTORS VEGFR-1 AND VEGFR-2 IN PATIENTS WITH LYMPHOID MALIGNANCY

D.W. Woszczyk, J. Gola, T. Gasinska, D. Ziaja, T. Urbanek, W. Kuczmik, U. Mazurek

Medical University of Silesia, Katowice, Poland

Backgrounds. Vascular endothelial growth factor (VEGF) is the most important proangiogenic factor involved in normal and pathologic angiogenesis. Biologic functions of VEGF are mediated by the activation of 3 structurally homologous tyrosine kinase receptors: VEGFR-1, VEGFR-2 and VEGFR-3. The exact role of VEGF receptors in the pathogenesis of lymphoma remains unknown. *Aims.* The aim of the study was to compare the concentrations of VEGF, VEGFR-1 and VEGFR-2 in the serum of 80 never-treated Non-Hodgkin's lymphoma patients in different stages of the disease [35 of these patients were diagnosed with the aggressive lymphoma and the remaining 45 patients with the indolent lymphoma; the control group consisted of 17 patients with persistent chronic lymph node enlargement). *Methods.* Serum VEGF, sVEGFR-1 and sVEGFR-2 levels were determined by means of the enzyme-linked immunosorbent assays (ELISA) (R&D Systems, USA) according to the manufacturer's protocol. *Results.* The VEGF serum concentration was found to be significantly higher in the aggressive lymphoma group when compared to the control group (median=433 pg/mL and 231 pg/mL, respectively; p=0,02026). In the indolent lymphoma group the VEGF concentration was also higher than in the control group, showing a tendency towards statistic significance (p=0,057392). There was no significant difference as far as VEGF between the two studied lymphoma subgroups. The serum concentrations of soluble VEGFR-1 were significantly higher in patients with both forms of lymphoma when compared to the control group (median=86 pg/mL and 44 pg/mL respectively; p=0,005318). The serum concentrations of the soluble form of VEGFR-2 were significantly higher in patients with the aggressive lymphoma when compared to the indolent lymphoma patients (median=10853 pg/mL and 8985 pg/mL, respectively; p=0,003501). We have not found a correlation between the serum level of VEGF and the soluble forms of its receptors VEGFR-1 and VEGFR-2 in any of the lymphoma patients. We have also checked the ratio as far as the amounts of VEGF and its soluble receptor (activity index VEGF/s-VEGFR-1). *Conclusions.* The results obtained for VEGF in patients diagnosed with the non-Hodgkin form of lymphoma confirm the role this protein plays in the pathogenesis of lymphoma, especially of its more aggressive form. The higher concentration of VEGFR-2 in the aggressive lymphoma patients when compared to the indolent lymphoma patients shows that - apart from the VEGF concentration - also the concentration of its receptors (especially its second receptor) has an influence on the course of lymphoma. This shows that not only VEGF but also its receptors should be the aim of the antiangiogenic therapy. To sum up, concentrations of VEGF and its VEGFR-2 may have an important influence on the course of non-Hodgkin's lymphoma.

Stem cells - Biology

0862

INCREASED MT1-MMP EXPRESSION IS INVOLVED IN G-CSF-INDUCED MOBILIZATION OF HUMAN CD34+ HEMATOPOIETIC PROGENITOR CELLS

A. Avigdor,[1] P. Goichberg,[2] Y. Vagima,[2] I. Petit,[2] M. Tesio,[2] I. Hardan,[1] O. Perl,[1] E. Rosenthal,[1] I. Resnick,[3] A. Nagler,[1] T. Lapidot[2]

[1]*The Chaim Sheba Medical Center, TEL-HASHOMER, Israel;* [2]*The Weizmann Institute of Science, REHOVOT, Israel;* [3]*Hadassah Univ. Hospital, JERUSALEM, Israel*

Backgrounds. G-CSF is the most established agent for hematopoietic progenitor cell mobilization in clinical practice. G-CSF-induced mobilization is initiated by activation of neutrophils, which secrete various matrix metalloproteinases (MMPs) and serine proteases. These soluble enzymes degrade bone marrow (BM) extracellular-matrix (ECM) and modulate cytokines and receptors, leading to a disruption of cell-cell and cell-matrix interactions and, ultimately, release of progenitors. Yet, progenitor mobilization by G-CSF was apparently normal in mice lacking these soluble enzymes. Therefore, we hypothesized that membrane type 1-matrix metalloproteinase (MT1-MMP), a membrane-bound MMP, might be also required for progenitor cell egress. MT1-MMP is a key enzyme for normal cell motility and tumor cell migration and invasion. Methods and Results. We found that human CD34+ cells express variable surface MT1-MMP levels, depending on the cell source and G-CSF treatment. The highest expression was found on CD34+ cells enriched from BM and mobilized peripheral blood (MPB) of G-CSF-treated donors (mean fluorescence intensity>900 and 159±40, respectively). MT1-MMP expression was lower in CD34+ cells isolated from the BM of untreated healthy human donors (80±19), and human cord blood (41±4). G-CSF treatment *in vitro* increased two-fold membranal MT1-MMP expression as compared to IL-6- or SCF-stimulated or untreated human CD34+ BM cells from healthy donors (steady state BM). Importantly, in vivo progenitor mobilization by five daily injections of G-CSF was accompanied by increased MT1-MMP mRNA and protein levels in both mouse BM mononuclear cells and human hematopoietic mature and progenitor cells in pre-clinical model of NOD/SCID mice engrafted with human hematopoietic cells. Immunocytochemical analysis of human CD34+ cells plated on hyaluronate-coated cover slips revealed that in response to SDF-1, MT1-MMP changes its localization in the polarized and spreading cells, suggesting a role in the process of progenitor directional migration. Indeed, blocking MT1-MMP function by antibody (Ab) or its endogenous inhibitor-TIMP-2 slightly but significantly reduced the *in vitro* chemotactic response of human MPB-derived CD34+ cells through uncoated transwell filters. The effect of MT1-MMP neutralization was even more prominent (60% inhibition) on the CD34+ cell chemotaxis via Matrigel, i.e., ECM barrier. Importantly, in vivo administration of human specific function blocking MT1-MMP Ab in the course of G-CSF treatment of NOD/SCID chimeric mice almost completely abrogated G-CSF mobilization of human maturing CD45+ leukocytes, immature CD34+ cells and the more primitive CD34+/CD38-/low progenitor cells. Finally, analysis of samples obtained from peripheral blood of 29 patients with lymphoid malignancies treated with chemotherapy and G-CSF revealed correlation between MT1-MMP expression level and the number of mononuclear cells and CD34+ progenitors on the day of first apheresis. *Conclusions.* we suggest that following G-CSF treatment, increased levels of MT1-MMP on the surface of human progenitors in the BM facilitates their mobilization most probably due to pericellular ECM degradation and/or activation of other regulatory molecules. Our data indicate that MT1-MMP plays an essential role in clinical mobilization procedures, and might serve as a target molecule for new approaches to enhance the mobilization efficiency.

0863

ROLE OF SONIC HEDGEHOG FOR REGULATING THE PROLIFERATION, MIGRATION AND DIFFERENTIATION OF HEMANGIOBLAST IN THE MICROENVIRONMENT OF AGM REGION

W.L. Liu, W.L. Liu, J.R Fu, M. Zheng, D. Ran, L. Luo, H.Y. Sun

TongJi Hospital, Tongji medical college, Wuhan, China

Background. Recently, it was reported that the intra-embryonic aorta-gonad-mesonephros (AGM) region exclusively and autonomously generated hemangioblasts for hematopoietic and endothelial system and dramatically increased hemangioblasts numbers thereafter. It is logically believed that the microenvironment of this region implicated in the

generation, maintenance, and perhaps even the expansion of hemangioblasts. But, untill now the mechanisms for regulating the proliferation and migration of hemangioblasts in AGM region were seldom reported. Aims To explore the role of sonic hedgehog (shh) for regulating the proliferation, migration and differentiation of hemangioblasts in the microenvironment of aorta-gonad-mesonephros(AGM). Methods The hemangioblasts were derived from AGM region of 11 days postcoitum (dpc) murine embryos with the CD34 positive and Flk1 positive immuno-magnetic beads. The phenotypic analysis of hemangioblasts and AGM-derived stromal cells were detected by flow cytometry. The secretion of shh was examined by immunohistochemical staining. The roles of shh in regulating the proliferation, migration and differation of hemangioblasts in the transwell non-contact coculture system co-cultured with AGM-derived stromal cells were observed by adding exogenous shh N-Terminus and its antibody. Results The protein of shh were observed highly expressed in AGM-derived stromal cells. The proliferation of hemangioblasts were promoted when co-cultured with AGM-derived stromal cells, and the effects could be blocked by antibody of Shh. The proliferation of hemangioblasts were strengthened further and could keep a long time without differentation and apoptosis when exogenous shh N-Terminus was added into the transwell non-contact co-culture system with AGM-derived stromal cells; While exogenous shh N-Terminus was added into the cultural supernatant of hemangioblasts without AGM-derived stromal cells, the hemangioblasts were observed to be induced to apoptosis or differentiation after a short time of proliferation. Furethmore, the ability of migration could be promoted in the co-cultured hemangioblasts by adding exogenous shh N-Terminus. *Conclusion*. Shh pathway could have an effect on the proliferation, differentiation, apoptosis and migration of hemangioblasts, but the roles were regulated by the microenvironment surrounding the cells.

0864

SONIC HEDGEHOG PROTEIN PROMOTE BONE MARROW-DERIVED ENDOTHELIAL PROGENITOR CELL PROLIFERATION, MIGRATION AND VEGF PRODUCTION VIA PHOSPHATIDYLINOSITOL 3-KINASE/AKT SIGNALING PATHWAYS

J.R. Fu, J.R. Fu, W.L. Liu, M. Zheng, D. Ran, H.Y. Sun

TongJi Hospital, Tongji medical college, WUHAN, China

Backgrounds. Recently, it was reported that shh pathway was involved in de novo vascularization of certain embryonic tissues as well as in inducing angiogenesis in an adult mammalian system. But if shh signal pathway plays a role in regulating EPCs behavior has not clear yet. Hence, the purpose of the present study was to address the question of whether shh protein effects BM-EPCs proliferation and migration, and then to further investigate the signaling mechanisms involved. *Aim*: To investigate the effects of Sonic hedgehog (shh) protein on bone marrow derived endothelial progenitor cells (BM-EPCs) proliferation, migration and vascular endothelial growth factor (VEGF) production, and the potential signaling pathways of these effects involved. *Methods.* Bone marrow-derived CD133+cells were enriched using the MACS system from adult bone marrow and then BM-EPCs were cultured in gelatin coated culture dishes. The effects of shh N-terminal peptide on BM-EPCs proliferation were evaluated using the MTT colorimetric assay. Cell migration was assayed using a modified boyden chamber technique. The production of VEGF was determined by enzyme-linked immunosorbent assay (ELISA) and immunofluorescence analysis. The potential involvement of PKC and PI3K signaling pathways was explored using selective inhibitor or western blot. 4 Results. The proliferation, migration and VEGF production in BM-EPCs could be promoted by endogenous shh N-terminal peptide at concentrations from 0.1 μg/mL to 10 μg/mL, and could be inhibited by anti-shh antibodies. Shh-mediated proliferation and migration in BM-EPCs could be partly attenuated by anti-VEGF. There exited low level of phospho- PI3-kinase in newly separated BM-EPCs, and the expression of phospho-PI3 kinase increased significantly when exogenous shh N-terminal peptide added , but could be attenuated by anti-human/mouse shh N-terminal peptide antibody. Moreover, the inhibitor of the PI3-kinase, but not the inhibitor of the PKC significantly inhibited the shh-mediated proliferation, migration and VEGF production. *Conclusion*. Shh protein can stimulate bone marrow-derived BM-EPCs proliferation, migration and VEGF production, which may promote neovascularization to ischemic tissues. This results also suggest that the angiogenic effects of shh involve in the PI3-kinase/Akt signaling pathways.

0865

DIFFERENTIAL EXPRESSION OF P-GLYCOPROTEIN, BUT NOT OF MRP, LRP AND BCRP IN LEUKEMIC STEM CELLS AS COMPARED TO MORE DIFFERENTIATED CD34+ AML BLASTS

L.L. Figueiredo-Pontes, M.C. Pintao, L.C.O. Oliveira, L.F.F. Dalmazzo, R.H. Jacomo, A.B. Garcia, R.P. Falcao, E.M. Rego

Medical School of Ribeirao Preto, Ribeirao Preto, Brazil

Leukemic Stem Cells (LSCs) retain the Hematopoietic Stem Cell properties of self-renewal, high proliferative capacity, predominant quiescent cell cycle status and differentiation potential, but is biologically distinct from more differentiated blasts. Quiescence confers protection from genotoxic stimuli to LSC, thus contributing to leukemia perpetuation. Currently, it is unknown whether the multidrug resistance (MDR) phenotype may also contribute to the LSC behavior. We analyzed the expression of the MDR transporters: P-glycoprotein (P-gp), MDR-Related Protein (MRP), Lung-Resistance Protein (LRP) and Breast Cancer Resistance Protein (BCRP) in LSCs from 20 CD34+ Acute Myelogenous Leukemia (AML) patients. We restricted our analysis to CD34+ cases, since high P-gp expression has been associated with this phenotype. Protein expression was measured by flow cytometry on LSCs, phenotypically defined as CD34+, CD38-, CD123+ cells, and compared to the more mature CD34+ CD38± CD123- blasts. The expression levels were analyzed using: 1) the Kolmogorov-Smirnov test, categorizing D value for descriptive purposes as high if $D > 0.30$, low if $0.2 < D < 0.3$ and negative if $D < 0.20$; and 2) the mean channel fluorescence index (MFI), defined by the ratio between the mean channel of fluorescence (MCF) for each antigen and MCF of the respective isotypic control. LSCs represented 0.12% (0.02-0.87%) of the leukemic blasts. We observed a direct correlation between MRP, LRP and BCRP D values in CD34+ blasts and LSCs. Based on the LSC analysis, 95%, 85% and 60% of AML patients were found to have high expression of MRP, LRP and BCRP, respectively. Whereas based on the CD34+ blasts, 90%, 80% and 60% of the patients were thus categorized. Accordingly, MFI for MRP, LRP and BCRP on the two cell subsets were not statistically different (MRP: 176.6 versus 153.3; LRP: 56.1 versus 71.3 and BCRP: 19.5 versus 22 on CD34 and LSCs, respectively). On contrast, P-gp expression was distinct in LSCs and CD34 blasts: the mean ± SEM of the D values were 0.38 ± 0.04 and 0.18 ± 0.04 (p=0.006), whereas MFI values were 22.9 ± 5.7 and 11.2 ± 5.9 (p=0.021) in LSCs and blasts, respectively. To our knowledge, this is the first study to show the overexpression of P-gp in the LSCs. Considering that P-gp-mediated drug efflux is the best characterized cellular mechanism of MDR, its constitutively high expression in AML LSCs may give rise to a genotoxic-protected stem cells theoretically capable of perpetuating leukemia

0866

IRON METABOLISM DURING HUMAN ERYTHROPOIESIS: EVIDENCE FOR A DIFFERENTIAL IMPACT OF TRANSCRIPTIONAL VS. POST-TRANSCRIPTIONAL CONTROL MECHANISMS

M. Samara, I. Chiotoglou, S. Lykousi, S. Samara, N. Stathakis, P. Liakos, P. Kollia

University of Thessaly Medical School, LARISSA, Greece

Iron homeostasis is critically modulated by the expression of transferrin receptors (TfR)-1 and 2 and ferritin. In most tissues TfR1 and ferritin expression is controlled by iron availability at the post-trascriptional level. The available data on regulation of iron metabolism in erythroid cells are limited and conflicting. In the present study, we evaluated TfR1/TfR2, ferritin H-/-L-subunit and iron response protein (IRP)-1/2 expression in a model system of human erythropoiesis. The objective of our study was to gain insight into the relative roles of transcriptional vs. post-transcriptional mechanisms in iron homeostasis during erythroid maturation. Peripheral blood CD34+ cells from normal individuals were cultured in serum-free StemSpan medium in the presence of stem-cell factor (100ng/ml) and erythropoietin (4u/ml). Real-time RT-PCR was used to quantitate TfR1, TfR2 (α/β isoforms), H- and L-ferritin mRNA levels. Western blotting experiments for TfR1/2, H/L ferritin and IRP1/2 were performed with appropriate antibodies on CD34+ cell lysates. Quantitative gel-banding densitometry was conducted on Epson GT-8000 Laser Scanner. TfR1 mRNA levels increased significantly during cell proliferation and erythroid maturation (4-7-fold over days 4-12). In contrast, TfR2-α mRNA transcripts were low at the proerythroblast stage, then increased x2-4-fold over days 4-12 and thereafter declined ~2.5 fold up to terminal differentiation. TfR2-β mRNA levels were also low in days 1-3 of culture and significantly declined throughout erythroid differentiation. TfR1 protein was detected from day 3; thereafter, a significant increase in TfR1 band intensity was observed during ery-

throid maturation (days 3-9), whereas a gradual decrease was observed after day 12. In contrast, TfR2 protein was undetectable at day 3; similar to TfR-1, TfR-2 band intensity peaked on day 9 and then gradually declined (especially after day 12). H- and L-ferritin mRNA levels peaked at day 3. Thereafter, a significant decline was observed throughout erythroid maturation; this decline was more pronounced for L-ferritin. H- and L-ferritin protein bands were first detectable after day 3 and peaked at days 6-9. Starting from day 3 up to terminal differentiation, IRP-1 band intensity increased x4-6 fold; IRP-2 was undetectable. These findings suggest that: (i) TfR2 mRNA isoforms are differentially expressed throughout erythropoiesis. (ii) TfR1 and TfR2 expression during erythroid maturation is controlled by transcriptional and post-transcriptional mechanisms, especially at more advanced differentiation stages; in contrast, H- and L-ferritin expression is mainly regulated at the post-transcriptional level. (iii) The abundance of IRP1 in erythroid cells and its upregulation during erythroid maturation are evidence of its important role in post-transcriptional regulation of both TfR1/2 and ferritin.

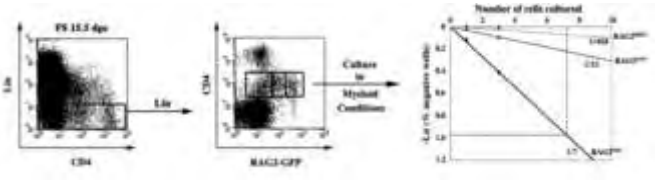
0867

THE SPLEEN AS AN EMBRYONIC HEMATOPOIETIC SITE: IDENTIFICATION OF FETAL SPLEEN PROGENITORS

E. Desanti, A. Cumano, R. Golub

Institut Pasteur, Paris, France

Backgrounds. During fetal life, the spleen is capable to sustain hematopoiesis. Contrary to the fetal liver and thymus, the fetal spleen (FS) contribution to hematopoiesis remains largely unknown. We have previously shown that FS stromal microenvironment does not sustain the hematopoietic stem cells (HSC) proliferation and multipotency. In contact with the FS stroma, HSC differentiate toward the myeloid lineage while their lymphoid engagement is prevented. However, the development of B cells is sustained in the FS when hematopoietic progenitor already possesses a lymphoid signature. *Aims.* To understand FS hematopoietic capacities, we identified hematopoietic progenitors that are present in the early stages of development (between 14.5 to 15.5 dpc). *Methods.* In the FS, we have isolated a CD4int lineage negative (Lin-) population by cell sorting and analyzed its *in vitro* and in vivo hematopoietic potentials. By limiting dilution assays and clonal assays, the frequency of B, NK, T and myeloid potentials were assessed. We tested the capacity of injected FS CD4int Lin- cells to reconstitute Rag2/gc-/- mice. By the use of RAG2-GFP mice, the CD4int Lin- population was further characterized. Moreover, by quantitative RT-PCR, we compared gene expression between FS CD4int Lin- population and other progenitors. *Results.* The FS CD4int Lin- population possesses lymphoid and myeloid potential *in vitro*. This population keeps its hematopoietic capacities in vivo since CD4int Lin- cells are able to reconstitute the lymphoid and the myeloid compartments of Rag2/gc-/- mice. The FS CD4int Lin- population could be subdivided into three subsets depending on the level of RAG2 expression (RAG2-, RAG2low and RAG2high). The RAG2- subset is mainly composed of myeloid precursors and we have shown that the loss of the myeloid potential is concomitant to the up-regulation of the Rag2 expression. By clonal assays, we displayed that the RAG2lo population is enriched by T/NK progenitors whereas the RAG2hi is mainly restricted toward the B lineage. After 4 days of FS organ cultures, CD4int cells are disappearing while lymphocytes are appearing suggesting that FS lymphocytes derived from in situ differentiation. Moreover, we have determined that the CD4int Lin- population are also the progenitors of the FS CD4hi lymphoid tissue inducer cells that may play a role in the FS architecture. *Conclusion*: The FS CD4int Lin- population encloses several progenitors that are engaged towards different lineages. These progenitors certainly give rise to committed hematopoietic cells *in situ*, indicating that the FS actively sustains the lymphoid.



0868

DIMINISHED PROTEASOMAL DEGRADATION RESULTS IN ACCUMULATION OF GFI1 PROTEIN LEVELS IN MONOCYTES

L.T. van der Meer, J.A.F. Marteijn, L. Emst, T. de Witte, J.H. Jansen, B.A. van der Reijden

University Medical Center Nijmegen, Nijmegen, Netherlands

Backgrounds. Gfi1 is a transcriptional repressor essential during myeloid differentiation. Gfi1-/- mice exhibit a block in myeloid differentiation resulting in the accumulation of an immature myelomonocytic cell population and the complete absence of mature neutrophils. Even though mRNA levels of Gfi1 appear to be very low in monocytes, Gfi1 might play a role in the monocytic lineage as Gfi1-/- mice exhibit diminished monocyte-derived dendritic cells and disturbed cytokine production by macrophages in response to LPS. *Aim.* Study the role of GFI1 in monocyte differentiation. *Methods*. GFI1 mRNA and protein levels were measured by qPCR and Western blot analysis respectively. Modifications of GFI1 with ubiquitin were analyzed with ubiquitination assays using His-tagged ubiquitin and binding of GFI1 to gene promoters was analyzed with chromatin immuno-precipitation assays. *Results*. Upon forced monocytic differentiation of U937 cells, Gfi1 mRNA levels dropped but protein levels increased indicating that Gfi1 protein expression is mainly regulated post-transcriptionally. To study this we performed ubiquitination experiments and found that Gfi1 is effinciently targeted by the ubiquitin-proteasome pathway. Treatment of cells with proteasome inhibitors MG132 or Velcade resulted in significant increases of both transfected as well as endogenous (U937) Gfi1 levels. Remarkably, after PMA induced monocytic differentiation of U937 cells proteasome inhibition did not result in an increase in Gfi1 levels. In line with this, we found that radioactive labeled *in vitro* translated Gfi1 was rapidly degraded in lysates taken from U937 cells, a process which could be blocked by proteasome inhibition. When lysates were taken from PMA stimulated U937 cells, the Gfi1 turnover was significantly delayed. Thus, during PMA forced differentiation of U937 cells Gfi1 protein levels rise due to diminished degradation. Similar findings were found in primary cells. Gfi1 mRNA levels were low in primary monocytes while the protein was clearly detectable. Conversely, Gfi1 mRNA levels were high in granulocytes but the protein was swiftly degraded by the proteasome in these cells. Chromatin immunoprecipitation experiments showed that Gfi1 binds to the promoter of several granulocyte-specific genes in primary monocytes, including C/EBPα, neutrophil elastase and Gfi1 itself. The binding of the repressor Gfi1 to these promoters correlated with low expression of these genes in monocytes compared to granulocytes. *Conclusions*. Gfi1 undergoes efficient ubiquitin-proteasomal degradation in immature hematopoietic cells. Upon monocytic differentiation proteins levels increase due to diminished proteasomal degradation, despite low RNA levels. Our data fit a model in which Gfi1 protein levels are induced in primary monocytes to repress genes that play a role in granulocytic differentiation.

0869

HIGHER CONSTITUTIVE NF-KB SIGNALING IS A DISTINCTIVE FEATURE OF UMBILICAL CORD BLOOD CD34+ PRECURSORS

R.A. Panepucci,[1] R.T. Calado,[1] V. Rocha,[2] R. Proto-Siqueira,[1] C.A. Scrideli,[3] R.C.V. Carrara,[4] A.R.D. Santos,[4] A.B. Garcia,[3] A.G. Araujo,[3] W.A. Silva-Jr,[1] M.A. Zago[1]

[1]*CTC-Fundherp FMRP-USP, RIBEIRAO PRETO, Brazil;* [2]*Hopital Saint-Louis, BMT Unit, PARIS, France;* [3]*FMRP-USP, RIBEIRAO PRETO, Brazil;* [4]*CTC-Fundherp, RIBEIRAO PRETO, Brazil*

Background. Delayed engraftment, better reconstitution of progenitors, higher thymic function, and a lower incidence of the graft versus host disease (GVHD), are characteristics associated with umbilical cord blood (UCB) transplants when compared to bone marrow (BM). These differences are in part due to the action of distinct genes and pathways among hematopoietic stem and progenitor cells (HSPC) of these two sources. *Aims.* We carried out a transcriptional analysis on human UCB and BM HSPC, in order to identify the molecular differences and factors responsible for their control. Methods. Pools of CD34-positive cells sorted by immunomagnetic methods (MACS) with over 92% purity were used to obtain RNA from HSPC of both sources. Transcriptional analysis was carried out by serial analysis of gene expression (SAGE). Differential expression of selected genes was evaluated by real-time PCR on additional CD34+ samples from BM (n=22), UCB (n=9) and G-CSF mobilized peripheral blood (MPB, n=6). *Results.* We sequenced approximately 60.000 tags from each SAGE library, roughly corresponding to

10.000 genes. Although HSPC from BM and UCB where very similar, a stringent statistical analysis revealed a set of 61 tags (transcripts) differentially expressed, 45 overrepresented in UCB and 16 in BM. The set of UCB-overrepresented genes included both subunits (NFKB2 and RELB) of the NF-KappaB transcription factor complex involved in the sustained activation of NFKB transcription targets, trough the non-canonical constitutive pathway. In addition, factors such as interleukin 1 (IL1A and IL1B), lymphotoxin B and receptors of the tumor necrosis factor family (TNFRSF1B, TNFRSF4); known to induce and sustain non-canonical NFKB signaling, were also found. Higher expression of transcripts coding for activators (IL1B, TNF and TGFB1), effectors (NFKB2, RELA and RELB) and transcriptional targets (ICAM1, IL8 and CCL4L) of NF-kB signaling were found in UCB HSPC and confirmed by real-time PCR. Finally, promoter analysis of the genes over-expressed in UCB HSPC revealed a statistically significant overrepresentation of NFKB cis-regulatory elements, including known NFKB transcriptional targets genes (such as CXCL2, CXCL3, ICAM1, IL8, IL1B, NFKB2 and RELB), and novel potential targets of NFKB signaling (like RGS1, zyxin and others). NOTCH1, which controls the transcription of NFKB, was also overrepresented in UCB. Conclusions. Our results point out to a central role of the NF-kB pathway on the molecular and functional differences observed between BM and UCB HSPC. Moreover, NFKB inhibition is known to cause apoptosis and loss of clonogenic function in HSPC. Furthermore, UCB HSPC readily differentiates into T cell on fetal thymic organ cultures, while BM HSPC must be pre-treated with TNF. Thus, NFKB transcription targets and other UCB overrepresented genes such as MIP1B, MIP2A, MIP2B, IL8, IL1, RGS1, zyxin, ICAM1, TGFB, LTB, TNF, may be responsible for the differences related to cell survival, quiescence, mobility and adhesion of these cells, as well as increased T cell diversity. The identification of this central mechanism sets the basis for future studies and to potentially new strategies to stem cell graft manipulation to improve the outcome of transplants with these cells, as well as their handling on propagation cultures.

0870

CLOSE FUNCTIONAL SIMILARITIES BETWEEN HUMAN MESENCHYMAL STEM CELLS, PERICYTES AND FIBROBLASTS

D. Covas, R.A. Panepucci, A.M. Fontes, M. Orellana, K.L. Prata, L.C. Pérez, A.G. Araujo, L. Neder, W.A. Silva Jr, M.A. Zago

University of S. Paulo, RIBEIRAO PRETO, Brazil

Backgrounds. Mesenchymal stem cells (MSC) are pluripotent precursors capable of differentiating into osteoblasts, adipocytes and chondrocytes, present in bone marrow (BM) and in various other adult and fetal tissues. MSC share many properties with pericytes, which form a continuous subendothelial network of the vascular bed, whereas the name stromal cell or fibroblast is often used interchangeably with MSC. Aims. To evaluate how similar are MSC obtained from different human tissues and what is their relationship with fibroblasts and pericytes on the basis of their gene expression and functional properties. Methods. A set of 30 genes was selected on the basis of previous results of serial analysis of gene expression (SAGE) for MSC, CD34+ cells and fibroblasts, and their expression was examined in 20 different human cell lines cultured *in vitro*: 7 MSC from different adult and fetal sources, 2 *in vitro* differentiated MSC cultures (one differentiated into osteoblasts and one into adipocytes), 4 of fibroblasts, and one of pericytes (isolated from the human retina), bulk bone marrow, endothelial cells, liver cells, brain tissue, skeletal muscle and heart tissue, respectively. Cells were characterized by their immunophenotype by flow cytometry, and the capacity to differentiate into osteblasts, adipocyte and chondroblasts. Gene expression of selected genes was measured by real-time PCR or semi-quantitative RT-PCR. Results. All MSC and the pericyte culture had the capacity to differentiate *in vitro* into adipocytes, osteoblasts and chondrocytes, whereas fibroblasts did not differentiated under similar conditions. Cluster analysis of gene expression profiles using Name 4.0 showed that all the MSC lines formed a very close cluster which included pericytes and fibroblasts, separated from other normal human cells. In addition, all the MSC lines and pericytes had similar immunophenotypic markers and capacity for *in vitro* differentiation. Similarity of the gene expression profiles of MSC and fibrobasts were further confirmed by clustering of the 1,000 top expressed tags of SAGE libraries for 21 normal human tissues. Despite the similarity, genes related to angiogenesis, especially CXCL6, were more expressed in MSC from umbilical vein, from adult saphena vein and in pericytes. Differentiation into adipocytes or osteblasts was accompanied by the increased expression of specific genes, although the global patterns were still very similar, so that they remained in the same cluster together with the MSC. Conclusions. MSC that can be obtained from a variety of adult and fetal tissues and pericytes have very similar biological markers, differentiation potential and gene expression profiles. Comparison of these characteristics also shows that human MSC, pericytes, and fibroblasts are very closely related, representing probably different functional states of the same cell. Identity between MSC and pericytes is particularly striking, whereas fibroblasts seem to have lost most of its differentiating potential. These results have practical as well as conceptual applications, since they demonstrate the functional equivalence of pericytes and MSC from different origins, and their close relationship to fibroblasts.

0871

IDENTIFICATION OF NOVEL REGULATORS OF HEMATOPOIETIC STEM CELL MOBILIZATION

C. Voermans,[1] W. Lento,[2] M. Uqoezwa,[2] L. DiMascio,[2] A. Chhotani,[2] F. Rattis,[2] T. Reya[2]

[1]*Duke Univ.Med.Center, USA / AMSTERDAM, Netherlands;* [2]*Duke University Medical Center, DURHAM, USA*

In an effort to identify the molecular changes during hematopoietic stem cell (HSC) regeneration we have analyzed genome wide changes in gene expression in HSCs in response to the chemotherapeutic agent cyclophosphamide (Cy) and the growth factor granulocyte colony stimulating factor (G). Cy/G treatment leads to an initial loss of proliferating precursors, followed by a rapid expansion of stem and progenitor cells and their subsequent migration to the peripheral blood. While this approach has been capitalized on for harvesting hematopoietic stem cells in clinical therapy, the regulation of this process remains less well understood. To analyze the molecular changes that occur as HSCs regenerate, we compared gene expression of HSC fractions from untreated mice and mice treated with Cy, G or Cy/G. Hierarchical cluster analysis of the data revealed that the molecular profile of Cy/G treated HSCs was most distantly related to that of untreated HSC compared to samples from Cy or G- treated HSCs. Additionally, among the genes upregulated by Cy/G, a large majority were a consequence of synergistic activity between Cy and G, while the rest of the upregulated genes could be attributed to activation by Cy or G alone. To test whether this screen allowed identification of novel genes that regulate HSC function we analyzed the role of transforming growth factor-β inducible gene-h3 (ßig-h3), a gene highly upregulated after Cy/G treatment. ßig-h3 is an extracellular matrix protein that mediates the adhesion and migration of various cell types; however its role in HSCs is unknown. Our experiments indicate that overexpression of βig-h3 in HSCs leads to accelerated differentiation *in vitro*. Consistent with this, transplantation of βig-h3-overexpressing HSCs resulted in reduced chimerism in vivo, and ultimate exhaustion of the HSC compartment. These experiments suggest that an enhanced ability to differentiate may be an important element of hematopoietic regeneration after injury, and that ßig-h3 is a regulator of such processes. These data also indicate that further functional analysis of other candidates identified through this comparative gene expression analysis will likely lead to identification of other novel regulators of HSC development and mobilization.

0872

DEVELOPMENT OF AN *IN VIVO* MODEL FOR THE STUDY OF TAL-1 LEUKEMOGENESIS AND T-ALL LEUKEMIC STEM CELLS

P. Ballerini,[1] F. Armstrong,[2] M.C. Rouyez,[2] P. Brunet de la Grange,[2] J. Landman-Parker,[3] P.H. Romo,[2] P. Ballerini,[4] F. Pflumio[2]

[1]*Hôpital Armand Trousseau, PARIS, France;* [2]*Inserm U567, Institut Cochin, Paris, France;* [3]*Onco-Hématologie, Hôpital Trousseau, Paris, France;* [4]*Hématologie Biologique-Hôpital Trousseau, Paris, France*

Background. T-cell acute lymphoblastic leukemias (T-ALL) are malignancies characterized by the abnormal production of T cell precursors, which are blocked in a differentiation stage. In 30% of the cases, leukemic cells express the TAL-1 oncogene by ectopic way. TAL-1 is a transcription factor, which is expressed very early during the embryogenesis and normally absent in mature lymphoid cells. However, following genic rearrangements, TAL-1 is expressed ectopically in thymocytes and this inappropriate expression is correlated to the development of a leukemic phenotype. The mechanisms of T cell leukemogenesis connected with the aberrant expression of TAL-1 are still unclear. Indeed, the enforced expression of TAL-1 is not sufficient for the initiation of the first stages of leukemogenesis. *Aims*. We developped an in vivo model of T-ALL in NOD-SCID mice, in order to understand the molecular

mechanisms implicated in T-ALL, and especially related to TAL-1. *Methods*. Four to five NOD/SCID mice, previously irradiated with 325 rads, were injected with 10 or 20 millions leukemic cells. When possible, positively separeted CD34+ cell precursors were also injected. Injections were done intra-veinously, intra-peritoneally or directly into the bone marrow. *Results*. We have transplanted 5 T-ALLs characterized by different oncogenic abnormalities (TAL-1, LMO2, HOX11L2) in NOD-SCID mice. So far 3/3 T-ALL samples induced a leukemia whatever was the injection route (intra-veinous, intra-peritoneal or intra-bone injection). Two experimental groups are still on going. Human leukemic cells were found in the peripheral blood, bone marrow, spleen, thymus and lymph nodes. However the spleen was the site, which contained the highest number of leukemic cells. In 2/2 T-ALL samples secondary transplantations were performed and induced a leukemia that allowed tertiary transplantation. The immunophenotype of the engrafted leukemic cells in primary and secondary mice was heterogeneous but still contained cells phenotypically identical to the original transplanted sample, especially when recovered from the mice bone marrow. In spite of the modification of the immunophenotype of leukemic cells, the serial transplantation results are evidences of the presence in the T-ALL samples of self-renewing leukemic stem cells. Transplantation of limiting numbers of total leukemic cells or of a CD34 positive leukemic cell population are on going in order (1) to quantify the frequency of T-ALL initiating cells in samples and (2) to try to delineate the phenotype of a leukemic stem cell population. *Conclusions*. This *in vivo* model will enable the study of the molecules involved in the development of T-ALL and particularly in the molecule that cooperate with TAL-1 in this pathology

0873

MESENCHYMAL STEM CELLS ARE EFFECTIVE AT PREVENTING BUT NOT TREATING GRAFT-VERSUS-HOST DISEASE

V. Tisato, K. Naresh, F. Dazzi

Imperial College, LONDON, United Kingdom

Background. Evidence has emerged that mesenchymal stem cells (MSC) represent a promising population for cellular therapy. MSC are of stromal origin and can differentiate into multiple lineages, including osteoblasts, chondrocytes, adipocytes, neurons and skeletal myocytes. MSC also possess immunosuppressive properties, which make them particularly attractive to control unwanted immune responses. For this reason they have been used in allogeneic haematopoietic stem cell transplantation to manipulate graft-versus-host disease (GvHD). We wanted to test the ability of MSC to prevent and /or treat GvHD in a xenogeneic model. *Methods.* After subletal irradiation, nonobese diabetic (NOD)/severe combined immune-deficient (SCID) mice were transplanted with 20×10^6 CFSE-labelled human PBMC obtained from normal buffy coats. Mice also received MSC generated from cord blood at the time of PBMC infusion or at the onset of xenogeneic-GvHD (x-GvHD). Recipient mice were evaluated at serial intervals for: 1. human T cells proliferation as measured by CFSE staining and number of CD45+/CD3+ cells; 2. clinical signs of x-GvHD (wasting, ruffled hair, hunched back). At the end of the experiment lymphoid and non lymphoid tissues were examined by histological analysis. *Results.* The human PBMC-NOD/SCID chimera monitored at different weeks after injection of PBMC showed extensive human T cells proliferation in their peripheral blood which was initially evident at 3 weeks. The mice started to develop signs of x-GvHD after 8-10 weeks and the disease was then confirmed by histology. Lymphoid infiltrates were evident not only in lymphoid tissues but also in liver, kidney, spleen, lung and peritoneal washing. The chimeric-mice injected with a single dose of MSC at the time of PBMC infusion did not behave differently form the controls. However, when MSC were given at weekly intervals, there was a marked decrease in human T cells engraftment and none of the mice developed x-GvHD. If MSC were administered when x-GvHD had already developed, no difference in T cells expansion and course of the disease was observed as compared to controls. *Conclusions.* Our study shows that systemic administration of MSC in human PBMC-NOD/SCID chimera dramatically increases the survival of the animals in a dose-dependent fashion. Human T cells proliferation and x-GvHD-induced tissue damage are markedly reduced in the treated mice and the use of MSC alone appears to be safe and well tolerated in this model. This work supports the clinical use of MSC infusion in SCT as a prophylactic treatment of GvHD.

0874

THE SIDE POPULATION MAY PROVIDE THE LEUKEMIC STEM CELL COMPARTMENT COMPLEMENTARY TO THE $CD34^+CD38^-$ STEM CELL COMPARTMENT. IMPLICATIONS FOR STEM CELL MRD DETECTION AND THERAPEUTIC TARGETING

B. Moshaver, N. Feller, A. Kelder, A. van Rhenen, G.J. Ossenkoppele, G.J. Schuurhuis, M. van der Pol, S. Zweegman, G. Westra

VU medical center, AMSTERDAM, Netherlands

Backgrounds. Acute myeloid leukaemia (AML) is a haematopoietic stem cell (HSC) disease. Although chemotherapy is initially successful in the majority of AML patients, many patients relapse, suggesting that present therapies are ineffective in eliminating the leukemic stem cells (LSCs). Although the AML $CD34^+CD38^-$ compartment is enriched for LSCs, not all LSCs can be $CD34^+CD38^-$, e.g. in CD34 negative AML. An alternative stem compartment is the so-called side population (SP). SP cells are defined by their ability to efficiently efflux Hoechst 33342 dye and in normal bone marrow (nBM) are enriched for HSC activity. In AML, the SP compartment is able to initiate leukaemia in NOD/SCID mice. The exact immunophenotype and the relationship with the CD34+CD38- stem cells are largely unknown. *Aim*: to define the immunophenotype of AML SP cells in relation to nBM SP cells. Such information may enable AML SP stem cell detection at all stages of disease/treatment and ultimately guide stem cell directed therapies. Methods. Using Hoechst dye and antibodies against CD34, CD38, CD7, CD19 and CD56 (the latter three offering leukaemia associated phenotypes, LAP used for MRD detection), as well as C-type Lectin-like molecule-1 (CLL-1), a marker for the AML $CD34^+CD38^-$ compartment (van Rhenen et al, Blood 2005; 106: 4), SP immunophenotyping was performed on BM samples of 8 AML patients and healthy donors. *Results.* The 8 AML patients had a median SP frequency of 0.01% (range 0.003-0.17%). For the immunophenotype, in terms of CD34 and CD38 expression, we found i) the whole blast compartment was partly $CD34^+CD38^+$ and partly $CD34^-CD38^+$ in the 5/8 CD34 positive (>1% CD34) cases and almost completely $CD34^-CD38^+$ in the 3 CD34 negative cases; ii) In 5 $CD34^+$ cases SP cells were in majority $CD34^+CD38^+$. The rest was mainly CD34-$CD38^+$; iii) also in the 3 CD34- cases $CD34^+CD38^+$ was the predominant phenotype (located in the very small $CD34^+$ compartment); iv) in all cases there was only a very small $CD34^+CD38^-$ compartment (median 4% of SP cells). LAPs present on the whole blasts were also present on the SP cells in all 6 LAP+ cases, indicating malignancy. SP cells from nBM samples were completely LAP negative. FISH analysis in a t(8;21) AML patient confirmed SP malignancy. In all 8 cases SP cells were partly or completely positive for CLL-1. SP cells from nBM were completely CLL-1 negative. *Summary/Conclusions.* Our results suggest that the phenotype of AML SP cells not necessarily reflects that of the whole blast compartment and in addition to being reported as $CD34^+CD38'$ or $CD34'CD38'$, in most cases is $CD34^+CD38^+$. CLL-1 and LAP expression on AML SP cells, similar to $CD34^+CD38^-$ stem cells, offers the ability for stem cell detection under MRD conditions and especially in those cases of AML missing the $CD34^+CD38^-$ stem cell compartment. In addition, CLL-1 expression on both AML SP and $CD34^+CD38^-$ LSCs and not on normal HSCs offers putative potential of toxin coupled antibody stem cell therapies now covering all known AML stem cell phenotypes.

0875

HUMAN UMBILICAL CORD BLOOD CELLS REGENERATE HEPATOCYTES IN A NON-MYELOABLATIVE SETTING

S. Wong,[1] G. Cheng,[1] K. Tsang,[2] D.F. Lau,[2] N. Chan,[2] W.S. Wong,[2] M.H. Ng,[2] C. Tong,[2] J.C. Tang,[3] C.H. Chui[3]

[1]*Prince of Wales Hospital, Hong Kong, Hongkong (China);* [2]*The Chinese University of Hong Kong, Hong Kong, Hongkong (China);* [3]*Polytechnic University of Hong Kong, Hong Kong, Hongkong (China)*

Backgrounds. A number of reports have shown that rodent bone marrow cells can transdifferentiate into hepatocytes. Human umbilical cord blood is a rich source of haematopoietic stem cells and mesenchymal progenitor cells which might be used for tissue or organ repair. Aims. We evaluated whether human umbilical cord blood cells infused following non-myeloablative conditioning can regenerate hepatocytes after acute liver injury in an immuno-competent mouse model. Methods. In an acute hepatic injury model, female C57Bl6 mice were administered toxic dose of acetaminophen. Six hours later, the mice were given fludarabine (0.5 mg/kg) and cyclosporine (3 mg/kg) followed by infusion of human umbilical cord blood mononuclear cells at a dose of 1×10^7 mononuclear cells per kilogram of body weight. The cyclosporine was

continued at 3mg/kg daily for four more days. Surviving mice were sacrificed at two and four weeks post transplant. Fluorescence in-situ hybridization (FISH) and polymerase chain reaction (PCR) analysis of hepatic DNA for α-satellite region of human chromosome 17 were used to confirm the presence of hepatocytes from human origin. Results. Fifteen out of 24 mice received umbilical cord blood cells infusion after non-myeloablative conditioning survived beyond two weeks compared with two of the 11 control mice (p= 0.027). The surviving mice were sacrificed at two weeks and four weeks post-transplants. Histological sections showed regenerating liver with hepatocytes of normal appearance comparing with the livers of the control mice showing extensive necrosis. FISH analysis confirmed the presence of human Y-chromosome positive cells in the hepatic sections of all but three of the surviving mice, the percentages ranged from 0.5-9%. PCR analysis showed that all except 3 mice (lanes 9, 12 and 13) showed presence of about 1.5-20% human DNA (Figure). In three mice (lanes 6, 14 and 15), about 10-20% of the hepatic DNA was of human origin. There was a concordance between the proportion of human DNA detected by PCR and the percentage of human Y-chromosome positive cells by FISH. Non-hepatic tissue (heart and kidney) did not contain human DNA. Conclusion: Our data suggested that human umbilical cord blood could repair acetaminophen induced acute hepatic injury in a non-myeloablative setting. Our model closely mimics the clinical UCB transplantation setting and should be further explored in a clinical setting. In the future, this may be an effective approach in the management of patient with fulminant hepatic failure waiting for orthotopic liver transplantation.

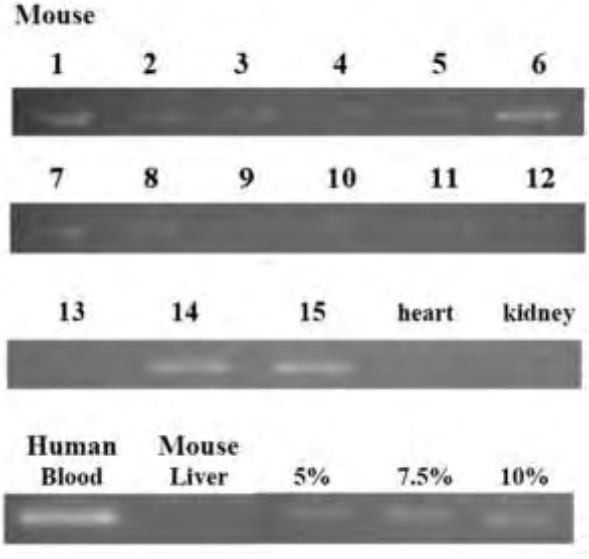


Figure 1. Detection of human DNA in the livers of mice survived.

0876

TOWARD STANDARDIZATION OF CELLULAR PRODUCTS FOR IMMUNOMODULATION AND REGENERATIVE MEDICINE. EXPANSION OF MESENCHYMAL STEM CELLS DERIVED FROM AMNIOTIC FLUID: PERSPECTIVES OF FUTURE CLINICAL APPLICATION

N. Sessarego,[1] M. Podestà,[1] A. Parodi,[1] M. Mogni,[2] A. Ibatici,[1] S. Pozzi,[1] F. Bertolotti,[1] M. Corselli,[1] F. Dagna-Bricarelli,[2] F. Frassoni[1]

[1]*Ospedale San Martino, GENOA, Italy;* [2]*Genetica Umana-Galliera, GENOA, Italy*

Background. Mesenchymal stem cells are now extensively studied in projects involving either their immunomodulatory property and their utilization in regenerative medicine. Because of the limited absolute number of bone marrow (BM) derived MSC available as cell therapy product we addressed our attention to MSC derived from alternative sources. Recently, it has been suggested that amniotic fluid (AF) is a rich source of MSC. Aim. In the current study, we evaluated the isolation and expansion of AF-MSC testing the immunophenotype and karyotype stability at different passages. The expansion of MSC derived from BM and AF were compared. Methods. Cell isolation from AF and BM. Second trimester samples of AF were centrifuged for 10 minutes at 400g. Cells were plated in Mesencult medium and, after 48 hours, non adherent cells were discarded. The expansion of AF-MSC was assessed plating MSC at four different density since the first passage. Bone marrow aspirates were obtained from posterior iliac donor volunteers. BM mononuclear cells (MNC) were isolated, plated in 75 cm^2 flasks in MesenCult medium and incubated at 37°C with 5% humidified CO2 atmosphere. Flow cytometry analysis: at each passage, surface expression of CD14-FITC, CD34-PE, CD45-PerCP, CD31-PE, CD73-PE, CD105-PE, HLA-I-APC, HLA-II-PE was determined. Results. MSC from AF could be extensively expanded in vitro showing immunophenotype similar to that of BM-MSC. Starting from 95 mL (median value) of BM aspirate we were able to obtain a median number of 23×10^6 MSC at first passage (P1) up to 36.34×10^6 MSC (range $4.6\text{-}90\times10^6$ and $9.9\text{-}144\times10^6$ respectively) at fourth passage (P4). Using the lowest plating density, we optimized the fetal MSC expansion, achieving the highest number of expanded AF-MSC (median number 1.2×10^9 and 2×10^{10} cells at P4 and P5 respectively). *Conclusions.* AF-MSC showed higher expansion capability when compared to BM derived MSC. The lowest cell plating density represents the best condition to promote AF-MSC expansion. Although this condition seems to boost cell growth, we didn't find any karyotype abnormality on all the samples tested. AF-MSC could represent a potentially very useful cell therapy product.

0877

INTRACORONARY AUTOLOGOUS BONE-MARROW STEM CELL TRANSFER AFTER MYOCARDIAL INFARCTION. PRELIMINARY RESULTS OF A RANDOMISED TRIAL

J. Perez-Oteyza, P. Ramos, M. Catalan Sanz, M. Perez-Abad, M.J. Blanchard, C. Heras, P. Cuevas, E. Asin

Hospital Ramon y Cajal, MADRID, Spain

Backgrounds. Recent experimental and clinical studies have shown that cardiac transfer of stem cells and progenitor cells derived from bone marrow may enhance functional recovery after acute myocardial infarction (AMI). Aims. To assess whether intracoronary transfer of autologous bone-marrow cells could improve left ventricular remodelling and global ejection fraction after 6 months' follow-up. *Methods*. A total of 40 patients with AMI, were randomly assigned to either a control group (n=20) that received optimum postinfarction medical treatment, or a bone-marrow-cell group (n=20) that received optimum postinfarction medical treatment and intracoronary transfer of autologous bone-marrow cells in the first week after the primary percutaneous coronary intervention. Autologous bone marrow stem cells were obtained by iliac crest aspiration. After density gradient centrifugation, mononuclear cells were incubated for 24h in Teflon bags with X-vivo medium at 37°C. Prior to intracoronary infusion, cells were washed and resuspended in 10 ml normal saline. We assessed left ventricular volumes and function from baseline to a minimum of 6 months' follow-up by cardiac magnetic resonance imaging. *Results.* Median volume of bone marrow aspirate was 36 ml (range 30-40), with a total number of mononuclear cells of 137 million (65-400). The number of infused cells was 80 million (20-215), with a viability of 90 ±12%, and a recovery rate of 59 ±19%. The content of CFU-GM and BFU-E was 45 ±21 and 150 ±106 per dish, respectively. No infusion related complications were observed. Global left ventricular ejection fraction (LVEF) at baseline was 43.7% ±14.2 in controls and 43.8% ±12.7) in the bone-marrow-cell group (p=0.26). Functional evaluation after 6 months is available so far in 23 patients (11 controls and 12 treated with bone marrow cells). Mean global LVEF in controls was 49.3% (p=0.25) and 46.15% (p=0.64) in the bone-marrow-cell group. No significant differences in ventricular volumes were found between both treatment groups. *Conclusions.* In patients with marked left ventricular dysfunction after AMI, we haven't found significant improvement of LVEF in the bone-marrow-stem-cell group after 6 months' follow-up. The potential impact on long-term survival needs further evaluation.

0878

LOCAL INJECTION OF BONE MARROW CELLS AUGMENTS THE NEOVASCULARIZATION IN A MOUSE ISHEMIC HIND LIMB BY INDUCING VEGF

J.A. Kim, C.H. Lee, B.S. Lee

College of medicine,Catholic University., Suwon, South-Korea

Backgrounds. Improved neovascularization is an important therapeutic goal after myocardial infarction and limb ischemia. In the recent years, increasing evidence suggests that bone-marrow derived circulating cells home the sites of ischemia and contribute to the formation of new blood, so the direct injection of bone marrow cells (BMCs) in the ischemic sites might augment angiogenesis and collateral vessel formation. *Aims.* We examined whether the BMCS might induce the angiogenesis as effectively as endothelial progenitor cells (EPCs) in a mouse model of hind limb ischemia and evaluated the expression of related molecules. Methods. BMCs and EPCs were obtained from C57BL/6. Unilateral hind limb ischemia was surgically induced by femoral artery ligation in C57BL/6 mice (control group; n=4), autologous BM-MNCs (Group 1; n=4, $1.8\times0.2\times10^7$/animal) and EPCs (Group 2; n=4, $1.1\times0.21\times10^6$/animal) were transplanted into the ischemic limbs after 10 days. After 4, 8, 12 weeks, the capillary/muscle ratios were evaluated. And VEGF, eNOS, ProMMP-9 and MMP-9 were assayed in tissue homogenates using western blotting. MMP-9 activity was determined using SDS-PAGE gelatin zymography, too. Results. Injected PKH2 labeled BMCS were observed for more than two months and the capillary/ muscle ratio elevated continuously in group 1. 12 weeks after transplantation the group 1, group 2 had a higher capillary/ muscle ratio (1.27±0.03 vs 0.82±0.12) than control (0.62±0.12, $p<0.05$) (Figure 1). In group 1 and group 2, the expressions of VEGF, eNOS, proMMP-9 and MMP-9 were up-regulated than control. The expression of MMP-9 was normalized within 14 days after BMCs injection while the elevated expression of VEGF was sustained after 12 months (Figure 1). So enhanced vascularization by BMCs is thought to be related with the upregulated expression of VEGF. *Conclusions.* This result suggested that direct local transplantation of autologous BMCs augments the neovascularization in ischemic tissues. And prolonged expression of VEGF could eventually participate in blood vessel formation.

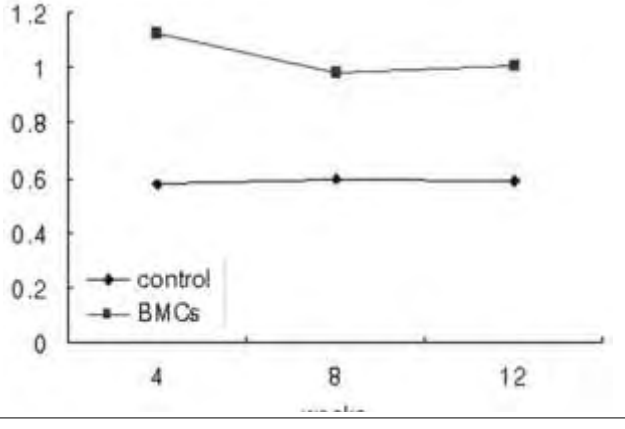


Figure 1. The expression of VEGF by IHC.

Vascular biology, granulocytes and infectious diseases

0879

ABNORMALITIES OF BONE MARROW MESENCHYMAL STEM CELLS IN PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA

E. Stavroulaki, M.C. Kastrinaki, I. Mavroudi, G. Eliopoulos, H.A. Papadaki

University of Crete School of Medicine, Heraklion, Greece

Backgrounds. Chronic idiopathic neutropenia (CIN) is an acquired underproduction neutropenia syndrome characterized by hypoplastic and left-shifted granulocytic series in the bone marrow (BM). Previous studies have shown that the bone marrow (BM) microenvironment may contribute to the pathophysiology of the disease by producing pro-inflammatory cytokines and pro-apoptotic mediators that result in defective support of granulocytopoiesis. Whether, however, there is a primary defect at the mesechymal stem cell (MSC) level in these patients remains unknown. Aims. To study the reserves, the functional characteristics and the differentiation potential of BM MSCs in patients with CIN. Methods. Thirteen patients with CIN and 22 age- and sex-matched healthy controls were studied after informed consent. All patients had neutrophil counts below 1800/microliter and were satisfying the previously reported diagnostic criteria for the disease. The BM mononuclear cells (BMMCs) were isolated from posterior iliac crest aspirates and the MSCs were expanded according to a standard protocol. MSCs were characterized by their immunophenotypic characteristics ($CD45^-$,$CD14^-$, $CD34^-$, $CD90^+$,$CD73^+$,$CD44^+$,$CD29^+$,$CD105^+$,$CD146^+$) and their adipogenic (Oil red O stain and aP2 and PPAR-γ expression by RT-PCR), osteogenic (ALP/Von Kossa stain and ALP and CBFA1 expression by RT-PCR), and chondrogenic (Masson and Alcian blue stain and Collagen II and Aggrecan expression by RT-PCR) potential after induction of differentiation in appropriate media.The frequency of MSCs in the BMMC fraction was evaluated by means of a limiting-dilution assay (LDA) based on the Poisson probability. The functional characteristics of MSCs were studied by evaluating (a) their clonogenic potential using a standard colony forming unit-fibroblast (CFU-F) assay and enumerating the CFU-Fs/100MSCs plated through passages (P), (b) their proliferative potential time-course by using the MTT assay and evaluating the cell doubling time (2°n=cells counted/cells plated) in each passage. *Results.* CIN patients displayed normal number (14.64±14.53 MSCs/10^5 BMMCs in the patients versus 23.78+/±6.49 MSCs/10^5 BMMCs in the controls; p=0.1986) and normal immunophenotypic characteristics of BM MSCs. The chondrogenic, osteogenic and adipogenic potential of patient MSCs did not differ from the respective of the controls as was evaluated by the collagen II and aggrecan, the ALP and CBFA1, and the aP2 and PPAR-_ mRNA expression, respectively, by means of a semi-quantitative RT-PCR. Compared to healthy controls, however, patient MSCs displayed impaired CFU-F potential time-course ($p<0.001$; P1-P6) as well as impaired proliferative capacity. This was demonstrated by the MTT assay ($p< 0.01$ at P1) and the cell doubling time time-course ($p< 0.001$; P1-P7). *Summary/Conclusions.* Patients with CIN display normal number and differentiation potential of BM MSCs. The clonogenic and proloferative potential of patient MSCs, however, is defective compared to the respective of the healthy controls. Analysis of the production of growth factors and inhibitors at protein level as well as the telomeric length of patient MSCs is currently under investigation to elucidate further the pathophysiologic basis of the observed MSC abnormalities in CIN patients.

0880

CONGENITAL NEUTROPENIA: A GROUP OF DISORDERS WITH GENETIC AND PHENOTYPIC HETEROGENEITY

C. Zeidler,[1] M. Germeshausen,[1] B. Schwinzer,[1] A. A. Bolyard,[2] G. Pracht,[1] B. Alter,[2] M.A. Bonilla,[2] B. Cham,[2] J. Donadieu,[2] C. Fier,[2] M. Freedman,[2] G. Kannourakis,[2] S. Kinsey,[2] L. Boxer,[2] D.C. Dale,[2] K. Welte[1]

[1]*Medical School Hannover, HANNOVER, Germany;*
[2]*SCNIR, WASHINGTON, USA*

Severe congenital neutropenia (CN) is a general term for a group of disorders characterized by extremely low blood neutrophil counts (ANC < 0.5×10^9), early stage maturation arrest of myelopoiesis, and recurrent

bacterial infections. In general more than 90% of CN patients respond to daily G-CSF treatment with a sustained neutrophil increase resulting in a significant reduction of infections and an improved quality of life. However, besides neutropenia, the presence of various concomitant clinical features in subpopulations of patients in conjunction with an increased risk of leukemic transformation in about 10% of all CN patients strongly suggested to search for new sub diagnoses to identify patients at risk for leukemia. The Severe Chronic Neutropenia International Registry (SCNIR) has collected longitudinal data on more than 400 patients with various causes of CN. This unique resource of data was used to classify different subtypes of CN, to estimate the relative frequency of these condtions and to correlate them to leukemic transformation. Our new classification scheme is as follows: 1. By inheritance - autosomal dominant, autosomal recessive, sporadic CN. 2. By genetic aberrations - *ELA2*, *GFI-1*, *WASP*, P14 related CN, SBDS related CN (Shwachman-Diamond syndrome), G 4.5-on-Xq28 related neutropenia (Barth syndrome), CXCR4 related neutropenia (myelokathexis and WHIM syndrome). 3. By clinical phenotype - associated symptoms (e. g. metabolic disorders, such as Shwachman-Diamond syndrome, glycogen storage disease 1b, Barth syndrome), G-CSF responsive or non-responsive CN, with or without acquisition of G-CSF-receptor mutations, with or without an evolvement of osteopenia/osteoporosis, or the presence of concomitant dysplastic features (e. g. organ abnormalities). Recent research and the work of the SCNIR lead to a significantly improved classification of CN. The identification of subtypes of CN, their distinctive risks of malignant transformation, and their responses to treatment has contributed substantially to our general understanding of the problem of neutropenia. This knowledge now also allows clinicians to provide patients and families with much more accurate prognostic information and better guidelines for therapy.

0881

SUPERIOR EFFECTS OF HIGH DOSE ENZYME REPLACEMENT THERAPY IN TYPE 1 GAUCHER DISEASE ON BONE MARROW INVOLVEMENT AND CHITOTRIOSIDASE LEVELS; A TWO CENTER RETROSPECTIVE ANALYSIS

M. de Fost,[1] C.E.M. Hollak,[1] J.E.M. Groener,[1] J.M.F.G. Aerts[1], M. Maas,[1] L.W. Poll,[2] M.G. Wiersma,[1] D. Hussinger,[2] S. Brett,[2] N. Brill,[2] S. vom Dahl[2]

[1]*Academic Medical Center, Amsterdam, Netherlands;* [2]*Heinrich-Heine University, Düsseldorf, Germany*

Background. Gaucher disease type I is the most common lysosomal storage disorder, caused by deficient activity of the enzyme glucocerebrosidase (OMIM *606463), leading to the accumulation of glucocerebroside in spleen, liver and bone marrow. The most important clinical manifestations are hepato and splenomegaly, cytopenia and skeletal involvement. Gaucher disease can be treated with enzyme replacement therapy (ERT), leading to a dramatic clinical response in most patients. Even after more than 14 years of experience, the most effective dosing regimen of ERT is still a subject of debate and varies from 15-130 U/kg/month, making a huge economic difference of 55.000 up to 300.000 Euro per patient per year. *Aim*. The aim of the study was to retrospectively compare long term outcome on hematological, visceral, biochemical and skeletal parameters in relation to two different dosing schedules (15-30 U/kg/4 weeks vs 80 U/kg/4 weeks). *Methods*. Adult Gaucher disease type I patients from two large European treatment centers, Amsterdam (AMC, N=49, median dose 15-30 U/kg/4 weeks) and Duesseldorf (HHU, N=57, median dose 80 U/kg/4 weeks) were included. Follow-up parameters included hemoglobin, platelet count, plasma chitotriosidase levels, liver and spleen dimensions, severe bone complications and scoring of bone marrow involvement by MRI of the femora. All parameters were matched at baseline and analyzed in two separate ways; comparison of baseline values vs values after one year and life table analysis (Kaplan Meier). *Results*. There were no significant differences in genetic background, age, gender, number of splenectomies and SSI in any of the matched populations. Improvement in hemoglobin, platelet count and hepatosplenomegaly was not significantly different between both cohorts, whereas bone marrow involvement by MRI, especially in patients with severe bone disease, and plasma chitotriosidase improved significantly faster in the higher-dosed group. Major bone complications rarely occurred in both groups. *Conclusions*. As improvement of hemoglobin, platelet count and liver and spleen volume is not dose-dependent, extensive organomegaly and cytopenia do not justify a high initial dose. The quicker response for bone marrow involvement upon a higher dose in severely affected patients is considered an important criterion to start a higher dose of enzyme. Chitotriosidase proves to be a sensitive indicator of dose effects and may be used in that respect to monitor response. The determination of the most cost-effective dosing regimen should be made individually and on the basis of a complete disease profile, including proper assessment of bone marrow involvement in addition to hematological, visceral and biochemical parameters.

0882

LUNG RESECTION FOR INVASIVE PULMONARY ASPERGILLOSIS IN NEUTROPENIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES: LONG TERM RESULTS IN THIRTY CASES

B. Neven,[1] C. Touzeau,[2] V. Roland,[2] S. Vigouroux,[2] S. Le Gouill,[2] B. Mahe,[2] V. Dubruille,[2] J. Delaunay,[2] P. Chevalier,[2] T. Guillaume,[2] C. Sagan,[2] F. Gay-Andrieu,[2] P. Germaud,[2] P. Despins,[2] P. Moreau,[2] J.L. Harousseau[2]

[1]*Hopital Necker-Enfants Malades, Paris, France;* [2]*Centre Hospitalier Universitaire, Nantes, France*

Invasive pulmonary aspergillosis (IPA) is a major cause of morbidity and mortality in neutropenic patients. Nevertheless, recent studies suggest that the outcome of IPA is improving due to early diagnosis (CT scan, antigenemia), use of new antifungal agents (*Azols* and *Echinocandins*), and possibly in some cases to early surgical resection. We here report a retrospective one center study of 30 cases of IPA treated by surgical treatment from 1988 to 2005. Patients were 18 men and 12 women, with a median age of 50 years (15 - 74). The underlying diseases were AML, ALL, aggressive lymphoma and myeloma in 22, 5, 2 and 1 cases, respectively. Surgery was planned after hematologic recovery from the last course of chemotherapy during which IPA was diagnosed, either possible in 15 cases, probable in 14 cases or proven in 1 case (Ascioglu, CID 2002). Surgery consisted in 1 pneumectomy, 4 bilobectomies, 17 lobectomies, 6 wedge resections and 2 lobectomies with wedge resections. No perioperative deaths occurred and the median duration of hopitalisation was 12 days. Four patients presented post surgical complications (pneumothorax, pneumopathy, section of phrenical nerve and bleeding). The diagnosis of definite IPA was confirmed in all 30 cases. Immediately after surgery, 24 patients were able to receive subsequent intensive chemotherapy courses, including 11 stem cell transplant (SCT), either auto (4) or allogenic (7). In all cases, patients subsequently received parenteral antifungal therapy. During these new intensive chemotherapy courses, recurrent aspergillosis was observed in only 2 cases, (inducing 1 death from brain localization). Overall, with a median follow-up of 8.8 years (1-18), 36% of the patients are alive and the main cause of mortality was relapse, but not IPA. In conclusion, early surgical resection together with antifungal therapy allows definite diagnosis of IPA, prevents from IPA recurrence and early death due to hemotysis, and at last allows subsequent high-dose chemotherapy to treat the underlying hematologic disease.

0883

THE ROLE OF DLL4 IN ENDOTHELIAL PROGENITOR CELL FUNCTION DURING TUMOR ANGIOGENESIS

C. Real,[1] R. Benedito,[2] A. Duarte,[2] S. Dias[1]

[1]*Instituto Portugus de Oncologia-Lisboa, Lisboa, Portugal;* [2]*CIISA, Faculdade de Medicina Veterinria, Lisboa, Portugal*

Backgrounds. In the adult, during tumour growth or in situations of vascular stress, angiogenesis is achieved not only by the recruitment of endothelial cells from neighboring blood vessels, but also by the mobilization of bone marrow (BM)-derived endothelial progenitor cells (EPCs). The differentiation and consequent incorporation of these circulating precursor cells into foci of neovascularization appears to be essential for the adequate assembly and function of the blood vessels. The Notch signalling pathway has been involved in vascular network remodelling and in arterial/venous identity of blood vessels during embryonic development. *Aims.* However, the function of this pathway in tumour angiogenesis still remains unclear, and is the subject of the present study. *Methods.* In this work, we first analyzed the expression of Notch signaling components in EPCs, isolated from mouse BM and in vessels that grow into xenografted human lymphoma. *Results.* Mouse EPCs expressed the receptor Notch1 and the ligands Delta-like 4, Delta-like 1 and Jagged 1. In addition, these cells also expressed the Notch downstream target gene Hey1, which was upregulated during EPCs differentiation *in vitro*. In situ hybridization analysis of the lymphoma xenograft showed that Dll4 expression level was higher and more frequently detected in the vessels than Notch1 and Notch4. Moreover, there was no arterial or venous restriction of the Dll4 expressing vessels within the

lymphoma xenograft. To evaluate in particular the role of Notch ligand Dll4 in EPCs function, we investigated the *in vitro* differentiation potential, cell migration and vessel network formation of EPCs isolated from Dll4± mutant mice. *Conclusions.* These results obtained thus suggest that the Notch signalling pathway might play a role in EPCs function, mediating the crosstalk between EPCs and endothelial cells during tumour angiogenesis.

0884

PRESENT TRENDS IN THE MANAGEMENT OF INVASIVE FUNGAL INFECTIONS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

H. Cherif, M. Kalin, M. Björkholm

Karolinska Institution, STOCKHOLM, Sweden

Background. Fungal infections are an important cause of morbidity and mortality in patients with hematological malignancies. Historically, treatment has been with amphotericin B and later its lipid formulations. However, new therapeutic agents have recently been introduced. The empirical use of antifungal therapy is today a standard approach in patients with persistent febrile neutropenia. However, the low incidence of invasive fungal infections (IFI) and the progress in the diagnoses and treatment of IFI have made the routine use of empirical antifungal therapy questionable. *Aims*. With the aim to define the present trends in the use of antifungal agents for the treatment of IFI we prospectively observed type, safety and efficacy of given antifungal treatment in patients with hematological malignancies during a recent 18-month-period. At the same time we analyzed the impact of restricted use of empirical antifungal therapy on IFI related mortality. *Patients and Methods*. Data was collected from patients admitted for treatment of febrile neutropenia to our department from November 2003 through April 2005. All patients who had received antifungal therapy empirically or for treatment of IFI were included. Local guidelines recommended a restricted use of empirical antifungal therapy to patients with persistent febrile neutropenia (5 days or more) , who were, after individual assessment, considered to be at high risk for IFI. Caspofungin was recommended for this indication. Voriconazole was recommended as primary therapy of invasive aspergillosis, while caspofungin in this setting was recommended as salvage therapy. *Results*. A total of 279 episodes of neutropenia and fever following chemotherapy were recorded. All patients were treated for hematological malignancies, predominantly acute leukemia (50%) . Treatment of IFI was given during the management of 41 (14%) episodes of febrile neutropenia occurring in 35 patients (Table). Voriconazole (27 episodes) and caspofungin (14 episodes) were the only antifungal agents used as initial therapy. Two patients received the combination of caspofungin and voriconazole as salvage therapy. The rate of antifungal therapy success outcome was 78% (Table). Oral preparation of voriconazole was given from the first day of treatment to 88% of patients treated with this agent. In general, antifungal agents were well tolerated and only two patients had to discontinue treatment due to severe adverse event. The overall 4-week mortality rate was 8%. Two patients died from invasive pulmonary aspergillosis. Empirical antifungal therapy was given to 13 patients with persistent febrile neutropenia without any signs of focal infection and resulted in successful outcome in 92% of cases. In 127 episodes of persistent febrile neutropenia antifungal therapy was deemed unnecessary and accordingly was not administered. In this subgroup of patients the overall 4-week mortality rate was 4% and 4 patients died of infection. No IFI related mortality occurred in this subgroup of patients. *Conclusions*. A better tolerability and efficacy of voriconazole and caspofungin together with the oral alternative of voriconazole have led to a shift in the use of antifungal agents for the treatment of IFI. A restricted use of empirical antifungal therapy was, in this setting, not associated with increased IFI related mortality.

Table 1.

	Number of episodes	*Successful outcome (%)*
Empirical therapy	13	12 (92)
Possible IFI	23	18 (78)
Probable IFI	4	1 (25)
Proven IFI	1	1 (100)
Total	41	32 (78)

0885

A SURVEY ON INVASIVE FUNGAL INFECTIONS IN SCT: TRENDS IN PROPHYLAXIS, TREATMENT, INCIDENCE AND CLINICAL OUTCOME AMONG 660 PATIENTS TRANSPLANTED DURING 2000-2004

J.F. Tomas,[1] D. Serrano,[2] J. de la Serna,[3] M. Canales,[4] J. Diaz-Mediavilla,[5] J.L. Lopez-Lorenzo,[6] S. Garcia,[3] F. Hernandez,[4] P. Garcia,[5] M. Callejas,[6] J.L. Diez-Martin[2]

[1]*Hospital MD Anderson, Madrid, Spain;* [2]*Hospital Gregorio Maranon, Madrid, Spain;* [3]*Hospital 12 de Octubre, Madrid, Spain;* [4]*Hospital La Paz, Madrid, Spain;* [5]*Hospital Clinico Universitario, Madrid, Spain;* [6]*Fundacion Jimenez Diaz, Madrid, Spain*

Backgrounds. Historical incidence (80s and 90s) of IFI in recipients of SCT ranges from 10-25% with an overall case fatality rate of up to 70-90%. Aims. Here we report our findings regarding the demographics, microbiology, clinical outcome and risk factors for the development of IFI among patients who underwent SCT in 5 hospitals of Madrid (Spain). Methodology: A retrospective study of all the patients who underwent SCT in 5 units of Madrid (Spain) during 2000-04 was done. Results. 120 patients received alloSCT (18%), 86 from a sibling donor and 34 from alternative donors. In 59 cases(49%) a RIC regimen was employed. PB was the source of stem cells in 650 patients (98%). Lymphoma(278) , acute leukemia(182) and myeloma(160) were the main underlaying diseases. 375 patients were in complete remission and 285(43%) were transplanted with persistent disease. 24 patients (4%) had a prior history of IFI. Determination of serum galactomannan was introduced in the last two years and data were available from 127 cases (20%). Nearly all patients received antifungal prophylaxis (639)(oral fluconazol in 576 cases-90%). An empiric antifungal treatment was instaurated in 190 cases(28%) and was more common in the allo population(40% vs 25%, p:0,007). Ambisome was the drug of choice in 120 cases (62%). IFI after SCT (EORTC criteria) ocurred in 32 patients(4,8%) (possible: 17, probable: 7, proven: 8). Median day of diagnosis was day +27(7-375), and pulmonary disease was the most common clinical presentation. Aspergillus was the most frequently involved mold(60%). 16/32 patients with a diagnosis of IFI have died (50%), in 5/16 cases death was attributed directly to IFI and contributed in other 4 cases. IFI was more frequent among allo vs auto(14,4% vs 2,7%; $p<0,0001$); AL vs other disease(7,6% vs 3,7%, p:0,04); a previous history of IFI (17% vs 4%, $p<0,02$); severe GVHD(grades III-IV and extense C-GVHD on IS treatment)(30% vs 4%; p:0,0002) and disease not in CR(6,67 vs 3,47;p:0,059). A multivariate analysis selected type of transplant(allo) (p:0,003; RR: 8,76), previous IFI(p:0,04; RR: 3,9), and severe GVHD(p:0,04; RR: 4,02) as the main risk factors for the development of IFI. Conclusions. Our findings shows that IFI had a low impact on mortality in the present series, 9/660(1,3%) and that current fatality rate among SCT with IFI was 28%(9/32) although global mortality in patients with IFI was 50%, higher that the non IFI cohort. Advances in clinical management, incluiding anticipate diagnoses, a more appropiate use of antifungal drugs, and the presence of low numbers of really high-risk patients could be argued for explanation.

0886

RANDOMIZED TRIAL OF PREVENTION OF CATHETER-RELATED BLOODSTREAM INFECTION IN PATIENTS WITH HEMATOLOGIC AND ONCOLOGIC DISEASE

A. Abdelkefi, W. Achour, S. Ladeb, T. Ben Othman, L. Torjman, A. Lakhal, A. Ben Hassen, A. Ben Abdeladhim

Centre National de Greffe de Moelle Osse, Tunis, Tunisia

Backgrounds. Data from the National Nosocomial Infection Surveillance system (United States) between January 1992 and February 1998 showed that Catheter-Related Bloodstream Infection (CRBI) is the third most frequent nosocomial infection and accounted for 14% of all nosocomial infections. CRBI may be caused by fibrin deposition associated with catheters. Interventions designed to decrease fibrin deposition have the potential to reduce CRBI. In a previous randomised study,[1] we have shown that the use of continuous infusion of low-dose (100 IU/kg per day) unfractionated heparin (UFH) was a practical and economical approach to the prevention of CRBI in patients with hemato-oncological disease. Aims. The purpose of this study was to evaluate the role of heparin-coated central venous catheter (CVC) in preventing CRBI in patients with hemato-oncological disease. Methods. This study was a randomised controlled trial in which patients were randomly assigned to receive either a heparin-coated CVC without a continuous infusion of low-dose UFH (heparin-coated group) or a non-coated CVC with continuous infusion of low-dose UFH (control group). CRBI was defined

according to the *difference in time to positivity*.[2] Results. Between April 2005 and February 2006, one hundred and twenty patients were randomly assigned. Two patients were excluded after assignment. Ultimately, 118 patients were analysed. CRBI occurred in 5% (3 of 59 catheters) of those in the heparin-coated group (2.2 events per 1000 days) and in 8.5% (5 of 59 catheters) of those in the control group (2.9 events per 1000 days) (p=0.7). Two and three patients experienced severe bleeding in the heparin-coated and control groups, respectively (p=0.5). We did not observe heparin-induced thrombocytopenia. Conclusion: The use of heparin-coated catheter is a safe and effective approach to the prevention of CRBI in patients with hematologic and oncologic disease.

References

1. Abdelkefi A, Torjman L, Ladebe S, et al. Randomized trial of prevention of catheter-related bloodstream infection by continuous infusion of low dose unfractionated heparin in atients with hematologic and oncologic disease. J Clin Oncol 2005;23:7864-70.
2. Abdelkefi A, Achour W, Ben Othman T, et al. Difference in time to positivity is useful for the diagnosis of catheter-related bloodstream infection in hematopoietic stem cell transplant recipients. Bone Marrow Transplant 2005;35:397-401.

0887

POLYMORPHISMS OF MBL2, BUT NOT OF FCGRIIA AND MYELOPERODASE PROMOTOR GENES, INFLUENCE THE RISK OF SEPSIS IN MULTIPLE MYELOMA PATIENTS DURING AUTOLOGOUS STEM CELL TRANSPLANTATION

I.M. Mølle,[1] N. Peterslund,[2] R. Steffensen,[3] S. Thiel[1]

[1]*University of Aarhus, Aarhus, Denmark;* [2]*University Hospital of Aarhus, Aarhus, Denmark;* [3]*Hospital of Aalborg, Aalborg, Denmark*

Background. A number of studies have indicated that polymorphisms of various immune defence genes interfere with the risk of severe infections in critically ill patients[1-3] including studies in autologous[4] and allogeneic[5,6] stem cell transplantation. In MBL2 three well-known single nucleotide polymorphisms B (G54D), C (G57E), and D (R52C)[7] (collectively termed O in contrast to the wild-type A) exist. Presence of O variants greatly reduces the effector functions of mannose-binding lectin - an important complement activating protein. The 131 H/R polymorphism of the Fcγ receptor IIA, located mainly on neutrophilics, strongly influence the binding capacity of the receptor, as the R variant binds to IgG2 poorly.[8] The G'463A polymorphism of the myeloperoxidase promotor gene is very sparsely studied as a risk factor for infections, but carriers of the A variant possibly are at increased risk of infections during allogeneic stem cell transplantation.[5] *Aims.* We aimed to examine the impact of the polymorphisms described above on the occurrence of severe infections related to ASCT in patients with multiple myeloma. *Methods.* Patients were genotyped with PCR techniques using aliquots of peripheral stem cells from apheresis. Infectious complications were recorded retrospectively from clinical records and database extractions from the Department of Microbiology. This was done blinded to the genotypes. *Results*. One hundred and eleven consecutive patients were studied. During ASCT 70 patients (63%) had fever above 38.5°C despite prophylactic antibiotics. Eleven (10%) patients had proven sepsis. MBL2 analyses: 4/71 patients with AA genotype had sepsis versus 7/40 with AO/OO genotype (p=0.09). Two lethal cases of sepsis were seen in the AO/OO patients, none in the AA patients. In multivariate analyses the risk of sepsis was significantly lower in AA patients: OR 0.15 (95% CI: 0.03-0.74), p=0.02. FCGRIIA and MPO promotor analyses: no association with fever or sepsis was found in patients with variant genotypes. *Conclusion*. Wild-type MBL2, associated with a high function of mannose-binding lectin, probably reduces the risk of sepsis in myeloma patients during ASCT. Myeloma patients with variant MBL2 may be candidates for future MBL replacement trials. In this setting we cannot confirm the protective effect of wild-type FCGRIIA or '463MPO genotypes suggested earlier.[5]

References

1. Sutherland AM, Walley KR, Russell JA. Polymorphisms in CD14, mannose-binding lectin, and Toll-like receptor-2 are associated with increased prevalence of infection in critically ill adults. Crit Care Med 2005;33: 638-44.
2. Gordon AC, Waheed U, Hansen TK, et al. Mannose-binding lectin polymorphisms in severe sepsis: relationship to levels, incidence, and outcome. Shock 2006;25:88-93.
3. Lehrnbecher T, Bernig T, Hanisch M et al. Common genetic variants in the interleukin-6 and chitotriosidase genes are associated with the risk for serious infection in children undergoing therapy for acute myeloid leukemia. Leukemia 2005;19:1745-50.
4. Horiuchi T, Gondo H, Miyagawa H et al. Association of MBL gene polymorphisms with major bacterial infection in patients treated with high-dose chemotherapy and autologous PBSCT. Genes Immun 2005;6:162-6.
5. Rocha V, Franco RF, Porcher R et al. Host defense and inflammatory gene polymorphisms are associated with outcomes after HLA-identical sibling bone marrow transplantation. Blood 2002;100:3908-18.
6. Mullighan CG, Heatley S, Doherty K et al. Mannose-binding lectin gene polymorphisms are associated with major infection following allogeneic hemopoietic stem cell transplantation. Blood 2002;99:3524-9.
7. Steffensen R, Thiel S, Varming K, Jersild C, Jensenius JC. Detection of structural gene mutations and promoter polymorphisms in the mannan-binding lectin (MBL) gene by polymerase chain reaction with sequence-specific primers. J Immunol Methods 2000;241:33-42.
8. Salmon JE, Millard SS, Brogle NL, Kimberly RP. Fcγ receptor IIIb enhances Fcγ receptor IIa function in an oxidant-dependent and allele-sensitive manner. J Clin Invest 1995;95:2877-85.

0888

DEVELOPMENT OF A FAST BROADBAND SCREENING SYSTEM FOR BACTERIAL BLOOD STREAM INFECTIONS USING WHOLE BLOOD AND CULTURE FLASKS

M. Handschur,[1] H. Karlic,[1] M. Pfeilstöcker,[2] E. Pittermann,[2] C. Disqué[3]

[1]*Ludwig Boltzmann Institute, Wien, Austria;* [2,3]*rd Medical Department, Hanusch hospital, Wien, Austria;* [3]*Molzym, Bremen, Germany*

Backgrounds. Bacterial blood stream infections are a common side effect in immune suppressed patients like cancer patients. Normal microbial routine diagnostics are time consuming and mainly based on classical cultivation. So far, molecular methods (e.g. PCR) were based on specific regions of specific microorganisms like Toxin-coding genes or species specific DNA fragments (e.g. 16S rDNA). We developed a novel extraction method where only bacterial DNA will be extracted and an amplification system that gives results within 2 hours. False positive results will be excluded and the infection causing organism will be identified at species level. *Aims.* Aim of this study was to detect the species of the microorganism in the blood stream as soon as possible after blood sampling. This may help to target an optimized antibiotic therapy and further medical treatments. False positive results had to be excluded. *Methods.* Blood of healthy donors and sterile culture flasks were spiked with various strains of bacteria known as causing clinical problems. The new extraction method only extracts the bacterial DNA. All human 'background' was digested. This increases the detection sensitivity and excludes false positive results attributed to cross reactions of bacterial universal primers with human mitochondrial DNA. By using PCR (Polymerase Chain Reaction) primers to amplify specific regions of the bacterial genome, PCR products vary in length specific to the amplified species: Each species gives a specific band pattern in the agarose gel after PCR- amplification. *Results.* Compared to commercial DNA- extraction kits, our extraction method shows a higher sensitivity and specificity when amplified directly from whole blood. No false positive results could be detected. PCR gives results 2 hours after DNA extraction. Using realtime PCR, 10^2 bacterial cells per ml blood could be detected. Performing a PCR after cultivation in a culture flask, each positive flask gave a positive PCR result. *Conclusion.* Our results show that the species causing the bacterial blood stream can be identified directly out of whole blood or a culture flask without sequencing the PCR product or further selective cultivation steps. Within 3 hours after blood sampling respectively 2 hours after culturing in the flask, a species specific therapy can be performed. The risk of false positive results was eliminated.

0889

31P MRS *IN VITRO* ASSAY OF PHOSPHOLIPIDS FROM PLASMA, PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC), AND BONE MARROW MONONUCLEAR CELLS (BMMC) OF PATIENTS WITH ACUTE LEUKEMIA (AL) AT THE TIME OF DIAGNOSIS

M. Kuliszkiewicz-Janus,[1] M.A. Tuz,[2] M. Kielbinski,[1] S. Baczynski,[3] B. Jazwiec,[1] I. Prajs[1]

[1]*Wroclaw Medical University, Wroclaw, Poland;* [2]*Inst. of Experimental Physics, U.Wr., Wroclaw, Poland;* [3]*Faculty of Chemistry, U.Wr., Wroclaw, Poland*

Backgrounds. 31P NMR spectroscopy is convenient and precise tool for the phospholipid analysis of extracts from biological samples. Previous investigations *in vitro* were performed on tissue extracts of solid carcino-

mas: breast, esophagus, colon, as well as on plasma of patients with thyroid and kidney cancers, multiple myleoma, malignant lymphoma. *Aims.* The aim of this investigation was to examine: (a) whether 31P NMR spectra of phospholipid extracts from plasma, PBMC, and BMMC are suitable for the analysis of phospholipid metabolism of blast cells from patients with AL; (b) whether obtained spectra allow to differentiate lymphoblastic acute leukemia (ALL) from myeloblastic acute leukemia (AML). *Methods.* 31P MRS spectra were obtained from phospholipids extracts of plasma (21 healthy volunteers, 44 patients with AL), PBMC (11 healthy volunteers, 52 patients with AL), and BMMC (38 patients with AL). Cellular phospholipids were isolated from mononuclear cells (MC) by means of Ficoll buffy coat centrifugation. Methanol-chloroform phospolipid extraction from 60×10^6 MC was performed according to the modified Folch's method. 31P MRS analyses were conducted on AMX 300 Bruker spectrometer 7,05 T. *Results.* 31P MRS spectrum of phospholipid extracts from normal human PBMC consisted of 8 peaks due to following phospholipids: phosphatidylcholine (PC), phosphatidylcholine plasmalogen (CPLAS), lysophosphatidylcholine (LPC), sphingomyelin (SM), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), cardiolipin (CL), and one due to MDPA. The peak due to LPC appeared only occasionally and not all spectra contained peak due to CL. However , the spectrum of phospholipid extracts from plasma consisted of 6 peaks due to phospholipids: PC, CPLAS, LPC, SM, PE, PI, and one due to MDPA. We observed , that spectra from phospholipid extracts from plasma and PBMC of patients with AL differed statistically from phospholipids of plasma and PBMC of healthy volunteers. Spectra obtained from phospholipid extracts of PBMC and BMMC patients with AL didn't differ. Spectra obtained from PBMC and BMMC of ALL patients differed significantly from AML patients. However, we didn't observe any statistically significant difference within spectra from plasma (Table 1).

Table 1. Concentration of phospholipids.

	ALL (nmol/l)	*AML (nmol/l)*	*ALL (nmol/l)*	*AML (nmol/l)*	*ALL (nmol/l)*	*AML (nmol/l)*
PC	2.01±1.58	1.62±1.06	0.32±0.18	0.40±0.18	0.24±0.16	0.42±0.26
CPLAS	0.05±0.08	0.06±0.06	0.02±0.03*	0.05±0.05*	0.01±0.02*	0.05±0.05*
LPC	0.08±0.11	0.07±0.09	–	–	–	–
SM	0.50±0.43	0.42±0.32	0.04±0.05*	0.09±0.06*	0.02±0.04*	0.11±0.09*
PI+PE	0.08±0.12	0.08±0.09	0.15±0.09*	0.28±0.17*	0.15±0.13*	0.30±0.19*
PS	–	–	0.01±0.02*	0.04±0.04*	0.01±0.02*	0.04±0.04*
CL	–	–	0.00±0.00	0.01±0.03	0.00±0.00	0.01±0.03

Conclusion. Our data show that 31P MR spectra from PBMC and BMMC are identic, both in position of peaks and concentrations of phospholipids. It may indicate, that blast cells from PB demonstrate the same metabolism of phospholipids as blast cells from BM. Only in PBMC and BMMC concentrations of CPLAS, SM, PI+PE, PS are significantly diminished in patients with ALL in reference to patients with AML. Explanation of reduction of mentioned phospholipides in lymphoblasts in reference to myeloblasts require further investigations.

0890

SAFETY OF A WEEKLY ADMINISTRATION OF 7.5 MG/KG OF LIPOSOMAL AMPHOTERICIN B FOR ANTIFUNGAL PROPHYLAXIS IN PATIENTS RECEIVING HIGH DOSE CORTICOSTEROIDS FOR ACUTE GVHD AFTER REDUCED INTENSITY CONDITIONING ALLOGENEIC STEM CELL TRANSPLANTATION

M. Mohty, J. El Cheikh, C. Faucher, S. Furst, H. De Lavallade, D. Blaise

Institut Paoli-Calmettes, Marseille, France

RIC regimens are increasingly used for allo-SCT in elderly or patients not eligible for standard myeloablative allo-SCT. Such regimens have yielded promising results in terms of decreasing early transplant-related toxicities. However, acute GVHD remains a matter of concern in this setting. Of note, the use of high dose CS for GVHD treatment increases the risk of severe fungal infections in this high risk population usually presenting several comorbidities. Therefore, prophylactic strategies aiming to reduce this risk are needed. We conducted a pilot single centre study in 15 adult patients receiving high dose CS (2 mg/Kg/day) for acute GVHD therapy after RIC allo-SCT. Treatment consisted of a 2 hour weekly infusion of 7.5 mg/kg LAB with a maximum of 8 total doses. The primary endpoint was the incidence of serious adverse events occurring during the course of prophylaxis treatment. Of note, safety was monitored with particular attention to nephrotoxicity in this relatively elderly population receiving concomitant nephrotoxic drugs such as cyclosporin A. Median age of these 15 patients with various hematological and non-hematological malignancies was 54 years (range, 40-70). Patients received a median of 4 weekly doses of LAB (range, 1-8), with 8 patients (53%) receiving at least 4 consecutive weekly doses. In terms of toxicity, 6 patients (40%) didn't experience any sign of toxicity. One patient experienced a violent chest pain with transient extra-systoles, during the first infusion of LAB, and did not receive any subsequent infusions. Other mild and transient infusion-related reactions (fever, flush, tachycardia, orthostatic hypotension, pruritous, bone pain, abdominal pain) were observed in 5 patients, usually at time of first LAB infusion. Despite concomitant administration of cyclosporin A in all 15 patients, only 4 patients (27%) had to interrupt the course of prophylactic LAB (respectively after 3 (n=2), 4 and 7 infusions) because of renal toxicity (increase of serum creatinine ≥1.5 times from baseline values). Although long term efficacy of such antifungal prophylactic strategy is yet to be established, the results of this feasibility study demonstrate that a weekly dose of 7.5 mg/Kg of LAB is relatively safe and well tolerated when given as prophylaxis in high-risk immuno-compromised patients receiving high dose CS for GVHD after RIC allo-SCT, despite concomitant administration of multiple nephrotoxic drugs.

0891

IMMUNITY AGAINST POLIO, DIPHTHERIA AND TETANUS AFTER CONVENTIONAL CHEMOTHERAPY TREATMENT FOR AML AND HIGH-GRADE LYMPHOMA

S. Einarsdottir,[1] P. Horal,[2] P.O. Andersson,[3] B. Kaijser,[4] V. Karlsson,[3] M. Brune[3]

[1]*Sahlgrenska University hospital, Goteborg, Sweden;* [2]*Department of Clinical Virology, Sahlgrenska University hospital, Sweden;* [3]*Department of Hematology, Sahlgrenska University hospital, Sweden;* [4]*Department of Clinical Bacteriology, Sahlgrenska University hospital, Sweden*

Background. In a recent paper, subprotective antibody levels against diphteria and tetanus were found in a majority of pediatric patients after treatment for high-risk acute lymphatic leukemia (Ek T *et al.* 2005). Information is scarce regarding immune reconstitution in adult patients who have undergone chemotherapy treatment for hematological malignancies. *Aims*. The aim of this study was to investigate whether protective antibody levels against diphtheria, tetanus and polio are retained in adults after intense chemotherapy treatment of acute myelogenous leukemia (AML) and high-grade non-Hodgkin´s lymphoma (HGNHL). Patients and Methods. Thirty-two patients, 18 males and 14 females, median age 61 (19-79) years, all in CR1 for a duration of >6 months after conventional treatment for AML (n=16) or HGNHL (n=16), were included. Twenty-nine healthy sex-matched persons, median age 60 (24-69) years, were enrolled as a control group. Immunity against polio types 1, 2 and 3 were assessed utilizing a standard neutralization assay, whereas antibody levels against tetanus and diphtheria toxoids were determined using an ELISA and a neutralization test, respectively. The minimum protective thresholds, as defined for clinical samples by the microbiology laboratory, were used to categorize patients and controls as immune or susceptible to infection. *Results*. Subprotective antibody levels against at least one of three polio serotypes were found in 10 out of 32 patients (31%), to be compared with 2/25 (8%) in healthy controls ($p<0.05$, χ^2 test). Twelve out of 32 (38%) patients versus 4/29 controls lacked immunity against diphtheria ($p<0.05$). With respect to immunity against tetanus, the difference between patients and controls (4/32 vs 1/29 pts) was not significant ($p=0.36$). *Summary*. We report a high prevalence of subprotective antibody levels against polio and diphtheria in AML and lymphoma patients who were in CR after conventional therapy not encompassing stem cell transplantation. Provided that these results can be confirmed in a larger study, assessment of immunity status, and possibly vaccination, may be considered not only in patients with leukemia and lymphoma but also in other patient groups receiving intensive chemotherapy.

0892

FLUOROCHINOLONE-RESISTANT ESCHERICHIA COLI IS THE MOST FREQUENT PATHOGEN ISOLATED FROM PATIENTS WITH HEMATOLOGIC MALIGNANCIES. RESULTS OF A PROSPECTIVE STUDY ON 364 CONSECUTIVE EPISODES OF FEVER AT A SINGLE INSTITUTION

C. Cattaneo,[1] G. Quaresmini,[1] S. Casari,[2] M.A. Capucci[1], M. Micheletti,[1] E. Borlenghi,[1] G. Rossi[1]

[1]*U.O. Ematologia, Spedali Civili, Brescia, Italy;* [2]*Istituto di Mal. Infettive e Tropicali, Brescia, Italy*

Background. Regular monitoring of the bacterial epidemiology at hematologic units has been recommended in order to evaluate the effects of the prophylactic and empiric antibacterial strategies adopted. Aims. To disclose the most frequent pathogens involved in infectious complications and the emerging resistance to antibiotics. Methods. We analysed all the consecutive febrile/infectious episodes occurring to the 823 patients admitted to our Institution (248 Acute Leukemia, 233 Lymphoma, 195 Myeloma, 26 Myelodysplastic Syndrome, 28 Chronic Lymphocytic Leukemia, 10 Myeloprolypherative Syndrome and 83 non neoplastic haematological patients) from June '04 to September '05. All the patients with expected neutropenia lasting for more than 7 days received prophylaxis with levofloxacin 500 mg/day. Results. Three hundred and sixty-four cases developed fever/infection (44.2% of all admission) and in 188 cases (51.6%) an infection was clinically documented (bacteremia 46%, pneumonia 42%, urinary tract infections 7%, others 5%). One hundred and sixty-four pathogens were isolated in 137 microbiologically documented infections (37.6%) including 82 Gram- bacteria (50%), 66 Gram+ bacteria (40.2%), 14 fungi (8.5%) and 2 miscellaneous (1.3%). E. coli, Enterobacteriaceae other than E. coli, and Pseudomonas spp were the most relevant Gram- strains (respectively 23.2%, 9.8% and 10.4% of all isolates). Among Gram+, S. aureus, Coagulase-negative Staphilococci (CoNS) and Enterococci were the most frequent pathogens, accounting respectively 15.2%, 11% and 7.9% of all isolates. E. coli was statistically more frequent in patients affected by acute leukaemia (26/69, 38% vs 10/68, 15%, p<0.01), neutropenia <500/mm3 (31/81, 38% vs 7/56, 13%, p<0.01) and on prophylaxis with levofloxacin (28/66, 42% vs 8/43, 19% p<0.05) S. aureus was associated with a non controlled underlying disease (24/91, 26% vs 1/46, 2%, p<0.01), neutrophil count >500/mm3 (20/56, 36% vs 5/81, 6%, p<0.01) and absence of prophylaxis with levofloxacin (11/43, 26% vs 3/66, 5%, p<0.01). Presence of central venous catheter (CVC) did not significantly predispose to CoNs infections (16/98, 16% vs 2/39, 6%, p=NS). Gram- bacteria showed resistance to Fluoroquinolones (FqR) in 42/61 cases (68.9%) associated by multivariate analysis only to prophylaxis with levofloxacin (OR 9.88, IC 2.32-42.01, p=0.002). Specifically FqR-resistant E. coli represented 91.7% of E. coli isolates (33/36). Hence it represented the pathogen most frequently isolated (21.3% of all isolates) among hematooncologic patients admitted to our Institution. Sixteen of the 25 (64%) Staphilococcus spp showed resistance to methicillin (MR), which was associated by multivariate analysis to prophylaxis with levofloxacin (OR 15.68, IC 1.03-238.13, p=0.047) and to CVC (OR 20.23, 1.34-305.69, p=0.03), but not to hospitalisation. Conclusions. In contrast with the recently reported prevalence of Gram+ bacteria in most haematological units, a shift toward Gram- bacteria, particularly FqR E.coli, was observed in our Institution. The role of levofloxacin prophylaxis in changing the epidemiological pattern and inducing FqR and MR needs further investigation.

0893

SIGNIFICANCE OF ENDOTHELIAL MICROPARTICLES, PLATELETS, AND LEUKOCYTE ACTIVATION IN PATIENTS WITH ACUTE CORONARY SYNDROME

M. Korzh, I. Kotchuev

Kharkov Medical Academy, Kharkov, Ukraine

Backgrounds. The details of interactions between endothelium, platelets, and leukocytes in ACS are not well understood. Aims. The purpose of this research was to determine the levels of platelet, leukocyte, and endothelial activation and markers of cellular interactions in patients with acute coronary syndrome (ACS). Methods. We studied 55 patients with VTE and compared 55 healthy controls. We used flow cytometry to measure: 1) endothelial microparticles (EMP) identified by $CD31^+/CD42b^-$ (EMP(31)) or E-selectin (EMP(62E)); 2) platelet microparticles ($CD31^+/CD42b^+$); 3) surface expression of P-selectin in platelets and CD11b in leukocytes; 4) EMP-monocyte conjugates (percentage of monocytes positive for E-selectin); and 5) platelet-leukocyte conjugates (PLC) expressed as percentage of leukocytes positive for CD41. *Results.* Patients with ACS had marked elevations of EMP(31) (2,213 vs. 372 counts/microl; p<0.01), EMP(62E) (379 vs. 231 counts/microl; p = 0.001), and EMP-monocyte conjugates (3.2% vs. 2.3%; p<0.01), as well as increased activation of platelets (31.9 vs. 4.8 fluorescence intensity units for P-selectin; p<0.001) and leukocytes (12.5 vs. 6.9 U for CD11b; p = 0.01). Also elevated in ACS were PLC (59.6% vs. 37.4%; p<0.01). Expression of CD11b in leukocytes strongly correlated with PLC (r = 0.64; p<0.001). Conclusions. Marked activation of endothelium, platelets, and leukocytes occurs in ACS, which involves the release of EMP and formation of EMP-monocyte conjugates and PLC. These findings support prior studies suggesting that release of EMP and their binding to monocytes are key events in thrombogenesis. Our findings also support the concept that the formation of PLC regulates leukocyte activation and participates in linking thrombosis with inflammation.

0894

CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN PATIENTS WITH ANCA-ASSOCIATED VASCULITIS

L. Kideryova, J. Zavada, R. Pytlik, P. Cervinkova, P. Klener, V. Tesar

1*st Medical Faculty, Praha 2, Czech Republic*

Introduction. There are two essential types of endothelial cells circulating in blood. First type are mature endothelial cells (CECs), which numbers are increased in microcirculation disorders such as ANCA associated small vessels vasculitis (AAV). These cells are connected with vascular damage. The other type are circulating endothelial progenitor cells (EPCs), which originate from bone marrow and play a crucial role in vascular repair and cancer neoangiogenesis. AIM. As there are several works studying mature CEC in patients with AAV, we have explored the frequency of immature EPCs in these patients. Methods. Circulating EPC numbers were determined in 35 patients with AAV, including 16 patients with newly diagnosed active disease without prior immunosuppressive treatment, 15 patients with active disease already treated by immunosuppressive therapy (pulse i.v. cyclophosphamide and peroral corticosteroids) and 10 patients in remission of the disease. Six patients were investigated twice (at diagnosis and in remission). We have used three groups of controls: 15 patients with non-AAV renal damage (patients on long-term hemodialysis), 9 patients suffering from macrocirculation disorders (ischemic disease of lower extremities) and 23 healthy volunteers. EPCs were enumerated by colony forming unit assay. 15-20ml of peripheral blood was centrifuged on Ficoll-Hypaque gradient (Pharmacia, Uppsalla, Sweden) and mononuclear fraction was cultivated in the EndoCultTM medium (StemCell Technologies, Vancouver, Canada) according to manufacturer instructions. Clusters of at least 20 central round cells surrounded by spindle-shaped cells were counted as endothelial precursor colonies. Results. The number of EPC was significantly lower in patients with AAV compared to healthy volunteers (median 0,5 vs. 12,3 EPC-CFU/ml blood, p<0,001). We did not find any statistical difference in numbers of EPC among groups of AAV patients before and after beginning of treatment and a group of patients in remission on maintenance immunosuppression. We have also found no correlation between the number of EPC and the Birmingham Vasculitis Activity Score (BVAS), level of C-reactive protein, plasma creatinine or titre of ANCA. Patients with ANCA anti-PR3 antibodies had a trend toward lower numbers of EPC compared to those with anti-MPO antibodies (median 0,18 vs. 3,15 EPC-CFU/ml blood, p=0,08). The number of EPC in patients on long-term hemodialysis was also significantly lower than in healthy volunteers (median 1,9 vs. 12,3 EPC-CFU/ml blood, p=0,001) and not statistically different from the number of EPCs found in patients with AAV. Patients with macrovascular disorders had non-significantly lower numbers of EPCs compared to healthy volunteers and significantly higher than patients with AAV (6,18 v. 0,5, p=0,035). CONCLUSION: Contrary to higher numbers of mature circulating endothelial cells, numbers of circulating endothelial precursors are significantly lowered in patients with ANCA positive vasculitis. This may reflect serious endothelial damage on one hand, coupled with diminished ability of endothelial healing. Immunosuppressive treatment, which is frequently also cytotoxic, may suppress not only the microvascular inflammation but also the endothelial healing process. Low numbers of EPCs in patients with terminal kidney disease may reflect the accelerated atherosclerosis found in uremia.

Funding: supported by: IGA NR 8047/3, MSM 0021620807.

0895

A RANDOMIZED COMPARATIVE STUDY OF RESPONSE TO EMPIRIC AMPHOTERICIN B DEOXYCHOLATE ON DAY 4 OR DAY 8 OF FEBRILE NEUTROPENIA

P. Malhotra, A. Makkar, N. Varma, A. Chakrabarti, S. Kumari, S. Jain, S. Varma

PGIMER, CHANDIGARH, India

Background. Febrile neutropenic patients are at greater risk of getting bacterial and fungal infections. Initial therapy for these patients consists of broad spectrum antibiotics. Persistent fever without localisation in spite of more than 3 days of broad spectrum antibiotics including vancomycin qualifies for initiation of empiric antifungal therapy. However, the timing of initiating empiric antifungal therapy can vary from 3 days to 8 days of non response to antibiotics. *Aims*. We choose to determine the response of empiric amphotericin B deoxycholate starting either on day 4 or day 8 in febrile neutropenic patients not responding to broad spectrum antibiotics and without localization of fever. The study also examined the side effects related to amphotericin B deoxycholate in this group of patients. Methods. Fifty six neutropenic patients with persistent fever despite 72 hours of antibacterial therapy were randomly assigned to receive amphotericin B either starting from day 4 (group A, n=27) or starting from day 8 (group B, n=29). Patients in both the groups were evaluated for efficacy and safety of study drug by the clinical criteria, frequent cultures, radiological procedures and laboratory parameters. A response was defined as satisfactory at the end of therapy if the patient was afebrile for 48 hours, had absolute neutrophil count (ANC) $> 0.5\times10^9$ /L, and did not require study termination due to patient's withdrawal from the study, drug toxicity, and persistent fever requiring change in therapy or death due to any cause. *Results*. The median age of patients in group A and in group B was comparable (23 versus 25 years). There were 17 males in group A and 18 males in group B. The patient population consisting of acute myeloid leukemia, acute lymphoblastic leukemia, aplastic anemia, non Hodgkin lymphoma, Hodgkin disease, chronic myeloid leukemia and multiple myeloma was equally distributed in two groups. A satisfactory response occurred in 85.2% of patients in group A and 69.0% of patients in group B (p=0.209). Time taken for resolution fever was considerably less in group A as compared to group B (5.4±3.9 days versus 11.3±4.0 days, p=0.0001) which was also reflected in total dose requirements between groups A & B respectively (592.8±258.4 mg versus 790.7±370.3 mg, p=0.028). The factors (age, sex, body mass index, baseline temperature, diagnosis, ANC) affecting the satisfactory response rate to amphotericin B were not statistically significant in two groups. Documented fungal infections were seen in 4 patients (14.8%) in group A as compared to 11 patients (37.9%) in group B (p=0.072). The adverse side effects of amphotericin B (nephrotoxicity, hypokalemia, hypomagnesemia) occurred at similar rates in the two groups. None of the risk factors studied (age, sex, body mass index, total dose of amphotericin B, baseline renal functions or exposure to various nephrotoxic antibacterial antibiotics) could be implicated in the causation of nephrotoxicity due to amphotericin B. *Conclusions*. We conclude that initiating early empirical (day 4) amphotericin B deoxycholate in persistent febrile neutropenic patients leads to early response rate and decreased dose requirements of amphotericin B without increased risk of nephrotoxicity.

0896

THE NEURONOPATHIC SPECTRUM OF GAUCHER DISEASE: A SINGLE-CENTRE CLINICAL EXPERIENCE WITH 15 PAEDIATRIC PATIENTS

E. Mengel,[1] C. Duck,[1] R. König,[1] Y. Amraoui,[1] L. Arash,[1] S. Pitz,[2] M. Beck[1]

[1]*Childrens Hospital Gutenberg-University, Mainz, Germany;* [2]*Augenklinik Gutenberg-University Mainz, Mainz, Germany*

Backgrounds. Gaucher disease patients are deficient in activity of the lysosomal enzyme glucocerebrosidase. As a consequence, accumulation of glucocerebroside occurs in macrophages in tissues throughout the body. An array of multi-systemic disease manifestations develops in part as a consequence of secondary damage. In neuronopathic Gaucher disease, the central nervous system is also affected. *Aims.* We studied the clinical characteristics observed in a cohort of paediatric chronic neuronopathic Gaucher patients under our care in order to delineate the spectrum of systemic and neurological presentations. The data were compared with clinical data collected from another cohort of 20 paediatric patients with non-neuronopathic (type 1) Gaucher disease. *Methods.* All 15 neuronopathic patients were thoroughly assessed at an initial evaluation (detailed history, physical examination, assessment of developmental status, and measurement of haemoglobin, platelet count and biochemical disease markers). Spleen and liver volumes were assessed (sonography) and chest X-ray, plain X-ray of the pelvis and DEXA scanning of the distal ulna were performed. Patients underwent comprehensive neurological examination, including evaluation of strabismus, eye movements and brain auditory evoked potentials (BAEP). *Results.* Compared to our non-neuronopathic patients, chronic neuronopathic patients had significantly earlier diagnosis, significantly greater anaemia, and higher serum chitotriosidase activity, as well as ACE and lysozyme levels. Most of our patients with severe neurological involvement had pronounced splenomegaly in conjunction with bone and lung manifestations. A markedly higher degree of radiological evidence of pulmonary interstitial involvement and higher frequency of skeletal complaints were apparent. A considerable variety in types and combinations of neurological symptoms was observed. Prominent neurological abnormalities with early development of a saccade initiation failure was ubiquitous in our series and a combination of strabism and saccade initiation failure was common. BAEP abnormalities were also common. A large percentage of our patients had severe neurological disease with hyperreflexia and ataxia and multifocal and/or myoclonic epileptic manifestations. Strabism as the first clinical neurological manifestation were detected in approximately half of our patients. *Summary/Conclusions.* The majority of our neuronopathic Gaucher patients first had systemic manifestations of Gaucher disease and subsequently developed oculomotor abnormalities as the first neurological symptom. Therefore, we recommend that objective eye movement assessment is carried out in all children diagnosed with Gaucher disease. Presentation and progression of neuronopathic disease was remarkably variable and, in fact, each patient is unique. Although the rigid historical categorisation of neuronopathic Gaucher disease variants is still used by many, we are of the opinion that it fails to express the variability. Systemic disease presentations in chronic neuronopathic Gaucher represented a more severe, early progressive condition than in type 1 Gaucher.

0897

CAUSES OF INCIDENTAL NEUTROPENIA IN ADULTHOOD

C. Lima, E. Paula, T. Takahashi, S. Saad, I. Lorand-Metze, F. Costa

State University of Campinas, CAMPINAS, Brazil

Backgrounds. The incidental discovery of neutropenia during routine blood counting represents a common problem for clinicians. However, there are no reported data of systematic evaluations of adults with incidental neutropenia. *Aims.* We aimed to identify the causes of incidental neutropenia in adults. *Methods.* Ninety-seven adults with incidental neutropenia were submitted to a clinical and laboratory approach, including complete and serial blood counts, direct and indirect antiglobulin test, bone marrow smear and biopsy, assessment of folate, vitamin B12 and iron status, serum liver enzymes, serum proteins, serological exams for hepatitis B and C virus, cytomegalovirus, mononucleosis, human immunodeficiency virus and toxoplasmosis, detection of LE cells, antinuclear and anti-DNA antibodies and rheumatoid factor, dosage of free thyroxin and thyrotropin, chest roentgenogram, and abdominal ichnography. The diagnosis of neutropenia due to exposure to chemical agents was defined in individuals with a occupational exposure to myelotoxic chemical agents and hypocellularity of the granulocytic lineage in the bone marrow. The infectious, autoimmune, haematological and thyroid diseases, and nutritional deficiency-related neutropenia were defined when diagnoses of the diseases or condition were established by specific laboratory exams. The recovery of the neutrophil count with the resolution or control of the disease was also required for the identification of these categories of neutropenia. Ethnic neutropenia was defined in individuals of African ancestry, in whom neutropenia was also present in other relatives. Drug-related neutropenia was diagnosed in patients under treatment with drugs, in whom the neutrophil count reached the normal value when the treatment withdrawn. Cyclic neutropenia was characterised by regularly recurring episodes of neutropenia and the diagnosis of chronic idiopathic neutropenia of the adult (CINA) was established when none of the above mentioned causes was found. *Results.* CINA was identified in 34.0% of the individuals, neutropenia due to exposure to chemical agents in 16.5%, infectious diseases in 9.3%, autoimmune diseases in 9.3%, haematological diseases in 9.3%, thyroid disorders in 8.2%, ethnic neutropenia in 7.2%, drug-related neutropenia in 2.1%, cyclic neutropenia in 2.1%, and iron deficiency in 2.1%. Recovery or improvement of the neutrophil count was seen upon treatment or recuperation from infectious, autoimmune, haematological and thyroid diseases, and iron supplementation. Conclusions. We conclude that the evaluation of individuals with incidental neutropenia using

a structured approach may possibilize the identification of clinically silent diseases, and provide the opportunity for early treatment, avoiding complications of the diseases and consequences of neutropenia.

0898

LONG TERM FOLLOW-UP OF TYPE 1 GAUCHER DISEASE PATIENTS. - A RETROSPECTIVE ANALYSIS

J.M.C.S. Costa Santos, M.J. Costa, J.A. Carmo, J.M. Fajardo, J.J. Gomes Oliveira

Santa Maria Hospital, Lisboa, Portugal

Background. Gaucher disease (GD) is a rare familiar disorder. It is caused by mutations in the glucocerebrosidase gene that causes a deficiency in β-glucocerebrosidade activity. In result, cleavage of glucose from ceramide is impaired and glucose-ceramide accumulates within cells. Three clinical phenotypes are recognized. Type 1 is the non-neurologic variant commonly seen in Ashkenazi Jews, but also found in other ethnic populations. Type 2, the infantile neuropathic form, and type 3, the juvenile onset variety, cause neurologic deterioration and are much rarer. *Aims*. Considering our hospital is a reference centre for GD in Portugal, our aim is to revise our Type 1 GD Patients data and report on diagnosis, treatment and long term follow-up results in order to enhance the understanding of this rare disease and to assess patients and their response to therapy over time, with the ultimate goal of improving the clinical outcomes through the definition of specific strategies. *Methods*. The total GD Type 1 patients (14) diagnosed at our outpatient department was enrolled to this retrospective analysis. All but 2 patients have therefore been on ERT for at least 1 year and the longest follow-up is 9 years. Amongst our group,12 patients were allocated to ERT (Enzyme Replacement Therapy) and the remaining 2 to SRT (Substrate Reduction Therapy); according to the approved indication. ERT (IV infusion treatment) initial loading dose was 60 U/Kg every two weeks except for one patient whose disease severity leaded to a 120 U/kg dose to start with. SRT (oral treatment) dose was 100mg TID. *Results*. The examined criteria for all patients included treatment related improvement (symptoms, physical examination, haematological parameters and bone disease) and Serious Adverse Events (SAEs) incidence. We found only one patient to be positive to Imiglucerase antibodies although there was no related clinical impact. This analysis indicated that ERT was well tolerated, we had no SAEs reported concerning both symptoms and laboratory parameters and the main issue turned out to be treatment adherence specifically related to infusions. Due to SAEs, SRT had to be discontinued to one of the treated patients and later on it was decided to initiate ERT. *Conclusions*. In respect to ERT, this study confirms the literature data concerning clinical improvement and good tolerance. At this time point, we have no sufficient data to draw conclusions on SRT and the patient on treatment is apparently doing well. In conclusion, the methodology adopted at our centre for this patient population has to be considered as appropriate; furthermore, ERT impact on disease parameters also enables us to assess compliance; considering patients' health status interest, the level of drug investment and the future consequences of disease progression, we are currently analysing patient profiles and motivations in order to identify positive strategies to raise treatment (infusion) adherence.

Thrombosis II

0899

THE SAFETY AND EFFICACY OF CONTINUOUS DYNAMIC DOSE ANTICOAGULATION DURING CHEMOTHERAPY-INDUCED THROMBOCYTOPENIA

N. Polizzotto, S. Opat

The Alfred Hospital, PRAHRAN, Australia

Background. The optimal management of patients with haematological malignancies who require therapeutic anticoagulation for thromboembolic disease or prosthetic cardiac valves while receiving myelosuppressive chemotherapy has not been established. In particular, the role of anticoagulation during chemotherapy-induced thombocytopenia, with its attendant increased risk of bleeding, has not been prospectively assessed. *Aims*. To assess the safety and efficacy of a dynamic dosing strategy for continuous anticoagulation during chemotherapy-induced thrombocytopenia. *Methods*. Patients were treated between January 2000 and January 2006 at The Alfred Hospital, Melbourne; prospective assessment occurred for patients treated between January 2005 and January 2006. All were receiving myeloablative chemotherapy for a haematological malignancy, and required anticoagulation for radiographically proven venous thrombosis or prosthetic cardiac valves. Patients were anticoagulated with subcutaneous enoxaparin as follows: 1 mg/kg body weight twice daily (*Full dose*) while the platelet count was ≥50×10^9/L and 0.5 mg/kg once daily to a maximum of 40mg (*Reduced dose*) while the platelet count was <50×10^9/L. Enoxaparin was withheld if the platelet count could not be supported above 20×10^9/L; if there was bleeding; and for 12 hours prior to and following procedures. Platelet were transfused to maintain a count ≥20×10^9/L. Results. Ten patients were enrolled prospectively, and 45 assessed retrospectively. In 54 patients, the indication for anticoagulation was venous thromboembolic disease; the other had a prosthetic aortic valve. Three underwent allogeneic stem cell transplantation, and seven autologous transplantation; the remainder received other myeloablative multi-agent chemotherapy regimens. Detailed information regarding the delivery of anticoagulation was available for patients enrolled prospectively. The median number of days of thrombocytopenia <150×10^9/L was 19 (range 9'40) . Enoxaparin was delivered at full dose on 31% of thrombocytopenic days, at reduced dose on 63% of days, and withheld on 6% of days . Of the days where enoxaparin was withheld, 45% were the result of procedures; 20% bleeding; and 35% zother reasons, including refractory thrombocytopenia <20×10^9/L. Major bleeding rate occurred in 5.4% of all patients. In the prospective group, three patients experienced episodes of minor bleeding. No major bleeding was observed in this group. In the retrospective group, two patients developed major gastrointestinal bleeding requiring endoscopic intervention and transfusion while receiving reduced dose anticoagulation with platelet counts of 20'50×10^9/L. One developed bleeding requiring surgical intervention at a wound site when the platelet count was 19×10^9/L and enoxaparin had been withheld. Data regarding minor bleeding episodes were not available for patients in the retrospective group. No thromboembolic complications were identified in either group. *Conclusions*. The strategy employed here was not associated with excess bleeding. It was effective in preventing recurrence and extension of thromboembolic disease in patients with a range of haematological malignancies. We conclude that dynamic dose anticoagulation may be delivered safely and effectively during chemotherapy-induced thrombocytopenia.

0900

SURVIVAL IN MULTIPLE MYELOMA PATIENTS IS NOT AFFECTED BY DEVELOPMENT OR RECURRENCE OF THROMBOEMBOLISM

M.Z. Zangari, F.C. Cavallo, B.B. Barlogie, G.T. Tricot

Univ. of Arkansas for Medical Sciences, LITTLE ROCK, USA

Backgrounds. Patients with cancer who developed venous thromboembolism have a poor prognosis. *Aims.* We have analyzed the effect on survival of treatment-associated VTE and their recurrence in a homogeneous population of myeloma patients enrolled on our Total Therapy 2 protocol. Methods. 668 newly diagnosed myeloma patients were enrolled in a study, which included induction phase with VAD, DCEP, CAD and DCEP followed by tandem high dose chemotherapy and peripheral blood stem cells (PBSC) transplants. Patients were randomly assigned upfront to receive Thalidomide or not. Both arms received identical chemotherapy. Patients were followed and when clinically indicated underwent radiological studies to confirm a suspected VTE.

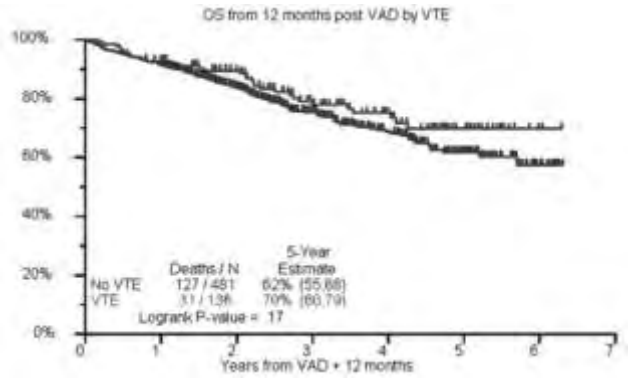


Figure 1. OS from 12 months post VAD by VTE.

Results. A total of 155 patients experienced VTE (median follow up of 47 months). Unbalanced baseline characteristics were balanced between patients experiencing VTE versus others were female gender , more frequent in the non VTE group on thalidomide, CRP > 8 mg/dl, and IL6 > 9 pg/mL were more frequently observed in the VTE group on no thalidomide. Within each arm of the trial no significant differences in prognostic factors for survival (chromosomal abnormalities, albumin level, β2-microglobulin, CRP) were seen. OS of the entire group by VTE status is shown (Figure 1). VTE recurrence was modest and not significantly different in those who resume thalidomide. *Conclusion*: Our experience shows that a thromboembolic event during treatment for myeloma patients does not affect survival.

0901

LOW-DOSE WARFARIN DECREASES THE INCIDENCE OF THALIDOMIDE ASSOCIATED VENOUS THROMBOEMBOLISM IN PATIENTS WITH MULTIPLE MYELOMA

K. Miller, S. Padmanabhan, L. Musial, D. DePaolo, A. Chanan-Khan

Roswell Park Cancer Institute, BUFFALO, USA

Introduction. T and its analog lenalidomide have established their role as novel antineoplastic agents. VTE is a common and often problematic side effect of these agents. The incidence of VTE increases substantially (up to 26%) when T is combined with other therapeutic agents such as dexamethasone (D), anthracyclin (A) or platinum compounds. Different approaches for T associated VTE prophylaxis have been explored without any consensus to date. Since the underlying pathologic event remains unknown, the optimal method for prophylaxis is also undefined. We prospectively investigated the use of low-dose warfarin for the prophylaxis of VTE in MM pts treated with T containing regimen. *Methods.* All pts treated with T containing regimens at our institute receiving prophylaxis with low-dose warfarin were evaluable. Warfarin was given at doses 1 or 2mg for actual body weight of < 70kg or > 70 kg respectively, which is continued for the duration of the T treatment. Concurrent aspirin or other anticoagulants were not given. Pts who were fully anticoagulated for a prior VTE or other VTE risk factors are excluded from this analysis. *Results.* Eighty-two consecutive MM pts, median age 60 years (range 43-82) who received low-dose warfarin for T containing regimen are reported here. T was given in combination with either dexamethasone, VAD regimen, Bortezomib (B) or bortezomib/doxil. Of these 38 are male and 44 female. Median dose of T was 200 mg per day (range 50-300). Duration of therapy with T varied with the regimen used with a maximum duration of 6 months. Of these pts, 54 (63%) received T with D containing regimen, 56 (68%) with A and 31 (38%) with B containing regimen. Some pts received multiple T containing therapies during their clinical course. Four (4.8%) out of 82 pts were noted to have a VTE. *Conclusion*. Although we continued to see VTE with T, our experience suggests that low-dose warfarin can effectively decrease the overall incidence of VTE in pts treated with T containing regimens. Interestingly, none of the pts who received T with doxil experienced any episode of VTE. These finding warrant further investigation of low-dose warfarin as VTE prophylaxis for T based therapies.

0902

PRESCRIPTION OF LOW-MOLECULAR WEIGHT HEPARIN FOR PROPHYLAXIS AGAINST VENOUS THROMBO-EMBOLISM IN MEDICAL PATIENTS

B. Myers, J. Wardle, J. Webb, J. Holmes

Queen's Medical Centre, University Hospital, NOTTINGHAM, United Kingdom

Thromboprophylaxis is vital to avoid thrombotic complications which may account for over 25,000 deaths per year in hospital admissions in the UK annually. A previous audit of medical thromboprophylaxis in 2004 in our institution, a large teaching hospital, demonstrated a poor level (22%) of appropriate prescribing. Evidence based guidelines were developed, disseminated and implemented in early 2005. A reaudit of the appropriate prescribing of thromboprophylaxis was then undertaken to establish the proportion of medical patients at risk of VTE correctly prescribed LMWH for thromboprophylaxis. Data were collected through a retrospective audit of medical notes and prescription charts. All medical patients admitted under a consultant physician in a week in April 2005 were included as long as their length of stay was greater than 24 hours. The audit replicated the methodology employed in the previous audit and utilised an existing audit tool. 196 patients were included. The median age was 72 (range 18-96) years. 179 (91.3%) patients were aged 40 or over. 52 (26.5%) patients in the sample were female. As documented risk assessment was poor (10%) satisfactory risk assessment was defined as those patients in whom appropriate thromboprophylaxis in a timely manner was prescribed. 51 patients were excluded from analysis when the thromboprophylaxis exclusion criteria were applied. Of 145 valid cases thromboprophylaxis was indicated in 34 (23.4%). Of 34 patients indicated as appropriate to receive thromboprophylaxis 19 (56%) were prescribed it. The remaining 15 patients were not prescribed thromboprophylaxis and in 14 cases neither a risk assessment nor a reason for omission was documented in the medical notes. Thromboprophylaxis prescribing rate has increased from 22% (2004) to 56% (2005). This is encouraging, but further improvement is required. A Trust Thrombosis Committee has been formed and a quarterly hospital-wide audit and a new thromboprophylaxis nurse specialist post are planned.

0903

CENTRAL VENOUS CATHETER-RELATED THROMBOSIS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND SCREENING WITH DOPPLER-ULRASOUND

M. Kaptan, A. Ifran, C. Beyan, O. Nevruz

Gulhane Military Medical Academy, ANKARA, Turkey

Backgrounds. Central venous catheters (CVCs) are vital component of care for patients with hematological malignancies. The use of CVCs provides an important means of venous access. CVCs are associated with the long-term risks of thrombosis and CVC-related thrombosis causes significant morbidity in the patients. However, not all of the thrombosis is symptomatic. *Aim*. To estimate the incidence of CVC-related thrombotic complications in hematological malignancies. *Methods.* We designed a prospective, observational study in our department. A total 78 CVCs in 42 consecutive patients with hematological malignancies were included in the study (males 28.6%, mean age 37.81 years + 2.89). Most frequent diagnosis was acute leukemia (87%). Three lumens catheters were inserted in the subclavian vein of all of the patients. A record of all complications and catheters loss and final out come were analyzed. Blood samples for thrombophilia screening tests were taken from all patients before insertion of CVCs. All patients underwent serial Doppler-ultrasound until CVC removal and we evaluated whether clinically manifest thrombosis could be predicted by screening with Doppler-ultrasound. Patients were clinically assessed each day for signs and symptoms of thrombosis. In case of clinically suspected thrombosis, routine diagnostic and therapeutic procedures were done. *Results.* A total 78 catheters remained in subclavian vein for a median of 43 days (range 4-140). Total CVCs related thrombosis was observed in 6.4% (5/78) of patients. Of the 5 patients with thrombosis, 3 had subclinical thrombosis by Doppler-ultrasound and no of them developed clinically manifest thrombosis later. Two patients had clinically manifest thrombosis without prior abnormal Doppler-ultrasound. Thrombosis of the catheters lumen (diagnosed upon the inability to aspirate blood with or without inability to flush occluded catheter) occurred in 20.5% (16/78). Catheter loss rate due to complication was 6.4% (5/78; 2 infection, 1 catheter thrombosis, 2 venous thrombosis). Neither total parenteral nutrition (p:0.46) nor difficult insertion of catheters (p:0.37) were related to thrombosis. Four patients had activated protein C-resistance (APC-R) and one of them had internal jugular vein thrombosis. *Conclusion*. The

incidence of clinically overt CVC-related thrombotic complications in patients with hematological malignancies is not negligible. The thrombosis of the lumen of the catheter is the frequent complication of central vein canulation. However their necessity of catheter removal is negligible. Although symptomatic disease was not developed in our cases of subclinical thrombosis, doppler-ultrasound screening may be useful to identify the patients with subclinical thrombosis that require antithrombotic treatment.

0904

DOPPLER ULTRASOUND ASSESSMENT OF SUBCLAVIAN VENOUS BLOOD-FLOW AFTER THE IMPLANTATION OF A CENTRAL VEIN CATHETER IN CHILDREN WITH MALIGNANCY

T.O. Ociepa,[1] E.M. Maloney,[1] E.K. Kamienska,[1] T.U. Urasinski,[1] M.S. Sawicki,[2] M.R. Rac,[2] A.W. Walecka[2]

[1]*Pomeranian Medical University, SZCZECIN, Poland;* [2]*Medical University, SZCZECIN, Poland*

A central vein cannulation is a routine procedure in the management of children with cancer. This long lasting, permanent venous access, resulting in a significant improvement of the quality of life of these patients is usually achieved by the implantation of a tunneled central venous catheter (CVC) into one of the subclavian veins. The undisputable benefit of this procedure is limited by its side effects, necessitating a catheter removal. In most of patients the clinical course of the procedure is uncomplicated however the late sequele of subclavian veins catheterization remain obscure. *Aim of the study*. This study aimed to assess venous blood flow in subclavian veins of pediatric cancer patients in which CVC had been previously implanted. *Patients and method*. The study comprised 37 children (13 girls and 24 boys, aged 3-18 years, median 9 years) with pediatric cancer (ALL-27, ANLL-4, Hodgkin's disease-5 and neuroblastoma-1 patient). In all of these children the central, tunneled catheter was inserted via the subclavian vein. The lumen of the CVC was rinsed once daily with heparin-lock (50 U/ml). The CVC clinically uncomplicated life span ranged from 124 to 620 days (median 249.9 days). Subclavian vein blood flow was assessed using a Doppler ultrasound (ATL HDI 3500; 11 MHz linear head) after CVC removal. The time from CVC removal to Doppler examination ranged from 1 to 54 months (median 23 months). *Results.* None of studied patients had clinical symptoms of subclavian vein blood-flow disturbances. Abnormal blood flow in the subclavian vein was found in 22 of 37 patients (59,46%). These abnormalities included: 1 signs of subclavian vein stenosis with no signs of thrombosis - in 11 of 37 (29,73%) patients, 2 signs of venous thrombosis - in 4 of 37 (10.81%) patients, 3 decreased pulsation amplitude and phase - in 7 of 37 (18.92%) patients. In the remaining 15 (40.54%) the subclavian vein blood flow was normal. The mean CVC life span in both groups of patients: with normal (n=15) and abnormal (n=22) subclavian vein blood flow did not differ significantly (275 vs 232; p=0.09). Conclusions. 1 A substantial proportion of children with uncomplicated course of central vein cannulation reveals the late ultrasound sequele of the procedure in form of subclavian vein stenosis and/or venous thrombosis. 2. The clinical relevance of these changes remains unknown and requires a long follow-up of larger groups of patients.

0905

TIME COURSE OF INFLAMMATION AND PROTHROMBOTIC PARAMETERS IN ACUTE CORONARY SYNDROME

O. Gutierrez,[1] A. Revilla,[2] E. Fontecha,[1] P.L. Sanchez,[2] M. Dueñas,[1] M.J. Pearrubia,[1] A. Cantalapiedra,[1] I. Gomez,[2] F. Fernandez-Aviles,[2] L.J. Garcia Frade[1]

[1]*Hospital U. Rio Hortega, Valladolid, Spain;* [1]*Icicor, Valladolid, Spain*

Background. The aim of this study is to assess the dynamics and magnitude of the thrombosis plasma markers in peripheral blood, and the relation to the degree of the inflammatory response across the spectrum of the acute coronary syndrome. Methods. Fifty patients with acute coronary syndrome; 10 with ST elevation myocardial infarction (STEMI), 10 patients with non-ST elevation myocardial infarction (NSTEMI), 10 patients with unstable angina (UA), 10 patients with stable angina (SA) and 10 comparable healthy controls were enrolled. The values of von-Willebrand factor antigen (vWF), thrombin-antithrombin complex (TAT) and prothrombin fragment 1+2 (F1+2) were assessed in peripheral blood using an enzyme linked immuno-assay method. The samples were collected on admission, daily during the first week, and days 14, 21 and 30 after the coronary event.

The inflammatory response was determined by the maximum levels of C reactive protein for every patient at the same times. Kruskall-Wallis test and Spearman´s Rho test were used to stablish relationship between these markers. Results. There were an increased prothrombotic response associated to myocardial damage, the values of vWF, TAT and F1+2 in patients with acute coronary syndrome were increased since the first day from the onset of symptoms, peaking on 4th or 5th day, and were detectable until 1 month. There were significantly higher levels in patients with myocardial infarction related to the other diagnostics (p< 0.01), specially at admission and at peaking plasma levels of these parameters (table 1). There were positive correlations between vWF & CPR (r= 0.67), TAT & CPR (r= 0.59) and F1+2 & CPR (r= 0.78), *Valladolid*< 0.001. *Conclusions*. This study demonstrates a marked prothrombotic response across the spectrum of acute coronary syndrome with correlation to the myocardial damage. The inflammatory response is closely associated with the prothrombotic response.

Table 1. Median values at admission (a) and maximum level (b).

	TATa µg/L	*TATb*	*F1+2a nmol/L*	*F1+2b*	*VWFa %*	*VWFb*	*CRPb U/l*
Control	2.2	3	0.48	0.5	101.5	106.5	1.75
SA	2.9	3	0.59	0.64	111.5	114	3.75
UA	2.5	15.4	0.75	1.12	102.3	138	43
STEMI	4.4	12.3	0.76	1.38	131.5	155	64
NSTEMI	7.6	14.1	1.03	1.66	140	153.5	107

0906

ELECTIVE SURGERY IN ANTICOAGULATED ATRIAL FIBRILLATION PATIENTS WITHOUT BRIDGING THERAPY WITH LMWH

M.A. Cortés, M.L. González-Ponte

Hospital de Laredo, LAREDO (CANTABRIA), Spain

Backgrounds. When oral anticoagulant therapy are discontinued for surgery, low-molecular- weight heparin (LMWH) is often used as bridging therapy. However, this practice has never been evaluated in randomized clinical trials. In our Hospital, the bridging therapy with LMWH is only used in prosthetic valves or embolism high risk atrial fibrillation patients. *Aims.* To assess the efficacy and safety of discontinuing anticoagulation in embolism low risk atrial fibrillation patients without bridging therapy. *Methods.* Retrospective observational study, from January 2000 to March 2005. Oral anticoagulant therapy was stopped 4 days before the elective surgery in atrial fibrillation patients without previous cardioembolism episode. If INR was >1.5 on the day of surgery, postponement was considered. When it was possible, oral anticoagulant was restarted the evening of surgery. Venous thrombosis prophylaxis was performed, when necessary, with dalteparin (5000 IU daily) starting 12 hours before surgery and until the INR was >1.9. Medical records (including emergency attentions) were reviewed to check arterial embolisms or bleeding episodes until 90 days after surgery. *Results.* 180 surgeries were eligible from 130 patients (73 men) with a median age of 76 years-old (44-91) and at least one of the following embolism risk factors: mitral valve disease, age >75, hypertension, diabetes mellitus or cardiac insufficiency. The majority procedures were ophthalmic surgeries (95), followed by orthopaedic, digestive system, urologic, gynaecological and otolaryngology surgeries (31, 24, 20, 9 and 1 respectively). 74% of the procedures were ambulatory major surgery, 23% major and 3% minor surgery. No surgery was postponed. In 35% of cases venous thrombosis prophylaxis was made. One patient (0.5%; 95% CI, 0 to 1.1) had a transient ischemic attack 21 days after surgery and 2 patients (1.1%; 95% CI,0.4 to 1.8) had an episode of bleeding (one mild metrorrhagia after gynaecological surgery and one acute cerebral hemorrhage 2 months after surgery). *Conclusions.* The arterial embolism ratio in our study is similar to the reported in anticoagulated patients when LMWH is used as bridging therapy. Oral anticoagulation treatment in atrial fibrillation patients without previous cardioembolism episode could be discontinued for surgical procedures without using LMWH bridging therapy . This strategy simplifies the periprocedural management of anticoagulation in these patients, reduces the cost and avoids LMWH adverse effects without increasing the embolic risk.

0907

VALIDATION OF THE COMPUTERIZED DECISION SUPPORT SOFTWARE TAOCHECK TO MONITOR ORAL ANTICOAGULANT THERAPY

M.A. Cortés,[1] E. Gómez,[2] A. Hervás,[2] R. Valero,[2] P. Muñoz[3]

[1]Hospital de Laredo, LAREDO (CANTABRIA), Spain; [2]Centro de Salud de Colindres, COLINDRES (CANTABRIA), Spain; [3]Gerencia de A. Primaria Santander-Laredo, SANTANDER, Spain

Backgrounds. Many clinical trials have demonstrated the utility of computer-based dosage programs to monitor oral anticoagulant therapy (OAT) in outpatients. However some of them are not already validated. Aims. We carried out a prospective, randomized trial to validate the effectivity of the computer aplication Taochek (Roche Diagnostics). Methods. From March to June of 2004, 118 outpatients on OAT in maintenance phase (more than 3 months under OAT) were randomized to two groups: 56 patients into the experimental group (Taocheck-aided dosing) and 62 into the control group (experienced physician dosing). There were not differences between two groups regarding age, sex, and diagnosis to AOT. Patients did not know the allocation. Dosing recommendations made by Taochek could be overridden by a physician. The comparison between both groups was performed by individual proportion of time spent within the therapeutic target INR range and the INR range ±0,3 by the mid-interval step method. Mann-Whitney test was used to contrast hypothesis. *Results.* There were no differences regarding the time in INR or INR±0,3 target range between groups: The patients of both groups spent the 50% of time into the INR target range (p=0.814); Taocheck group spent the 81.6% of time into the INR±0,3 target range vs 94.4% of control group (p=0.599). The number of appointments every 30 days in the Taocheck group was higher than in the control group (median 1.4 vs 0.9; p<0.001). From a total of 129 determinations in the Taocheck group, 6 times (5%) the dose was not proposed by the software or was overridden by the physician and in 5 time (4%) the first day schedule dose was modified. Conclusions. Our study demonstrated that OAT can be provided at least as well by computerized decision support software Taocheck as by experienced physician. However the Taocheck-aided dosing group required more number of appointments per patient than the control group. Taocheck software is useful for the AOT control and offers an effective help to inexperienced clinical staff.

0908

RETROSPECTIVE REVIEW OF INDICATIONS AND RESULTS IN 4000 CONSECUTIVE D-DIMER SAMPLES

B. Myers

Queen's Medical Centre, University Hospi, NOTTINGHAM, United Kingdom

D-dimers are used to assist diagnosis of venous thrombo-embolism (VTE), but are often inappropriately requested and interpreted. We retrospectively reviewed 4606 sequential d-dimer (DD) requests. We documented indication for request, repeat requests and compared results at different levels of raised DD. Our current DD test-kit is auto dimer (Trinity biotech), which has an upper limit of 180. Out of the 4606 requests, 2063 were 'negative' and 2603 were raised. The indications for DD requests were, in the large majority of cases, relating to diagnosis or exclusion of VTE. In a small minority of cases, the request related to disseminated intravascular coagulation (DIC). Indication for testing was obtained in those with raised DDs: chest symptoms (pain, dyspnoea; haemoptysis) comprised 4 3% (987 samples) and leg pain/swelling - 20% (460). There was a 20% positive VTE rate amongst these. However, a large number of requests were made for non-specific symptoms, and as expected, the test did not give useful diagnostic information in these instances. The range of level of a positive result varied from 181 to > 9999. We subdivided the positive results into six groups by extent of rise: > 8000; 4000-7999; 2000-3999; 1000-1999; 500-999 and 181-499. Within each group, the percentage of samples with a positive diagnostic imaging test for VTE was assessed. This percentage was remarkably constant at between 19-21%for all but the lowest level group, which had 12% confirmed VTE. Within the group of patients with VTE, we looked for evidence of active cancers. This was highest in the group with the highest DD levels, and declined through the groups, being respectively: 25%; 19%; 15%; 9%; 4.5%; 4.7%. In the small number of patients with levels over 8,000 (35 samples) a quarter of patients had cancer. This result is in keeping with studies demonstrating a correlation between very high DD levels and concurrent cancers, or diagnosis of VTE shortly predating cancer . Hence the finding of a grossly elevated DD should elicit an extensive search for malignancy, if not clinically evident. We also analysed numbers of patients having repeat tests within a very short time period. 42 duplicates were taken on the same day, 63 duplicates one day apart, 22 which were 2 days apart, 13 separated by 3 days, 8 at four and 9 at 5 days apart. In 2 patients, the test was requested (and performed!) on 3 samples on the same day. In only one instance did a repeat produce a different result, in a borderline positive case which became negative. These samples represent a very small percentage of the total workload, but were clearly not indicated. As is well-known, DDs rise during pregnancy and pregnant women often suffer dyspnoea, leg swelling or musculo-skeletal chest pain. Of 71 pregnant or postnatal women with symptoms and raised DD in all groups, only 2 had a confirmed VTE. In summary, DD is over-requested in our institution; grossly raised levels may have predictive value for cancer; defining separate ranges in pregnancy might be helpful

0909

ASPIRIN AND CLOPIDOGREL RESISTANCE, PLATELET C807T GLYCOPROTEIN IA POLYMORPHISM AND HYPERHOMOCYSTEINEMIA IN SURVIVORS OF MYOCARDIAL INFARCTION

I.G. Gaik,[1] E.H. Hanszke,[1] Z.T. Turowiecka,[1] M.D. Duszynska,[2] K.Z. Zawilska[1]

[1]University of Medical Sciencies, Poznan, Poland; [2]J. Strus Hospital, Poznan, Poland

Background. Recent studies suggest that among patients suffering from acute coronary syndromes there is a large group of patients resistant on acetylsalicylic acid (ASA) and clopidogrel therapy. Hyperhomocysteinemia and platelet C807T glycoprotein Ia (GPIa) polymorphism is common among reffered individuals. Apart from platelet glycoprotein IV (GPIV), GPIa is known as the main platelet subendothelial collagen receptor. *Aims*. The aim of the study was to find a relation between aspirin and clopidogrel resistance and GPIa polymorphism. *Material and methods*. The study group consisted of 50 patients aged 40-76 (median 57.2): 35 men (70%) and 15 women (30%). 32 of them had positive family history of coronary disease. All patients were treated with ASA (150 mg/24h) and clopidogrel (75 mg/24h) for at least one month after the coronary angioplasty performed because of acute coronary event. All patients had a diminished intraplatelet concentration of malonylodialdehyde (MDA) due to ASA ingestion. Resistance to antiplatelet drugs has been determined by the following criteria: the intensity of platelet aggregation induced by ADP >60%, by collagen >70%, and by arachidonic acid (AA) >20%. Platelet C807T glycoprotein Ia polymorphism has been detected by allelospecific PCR/RFLP method (Santoso S. *et al*.). Homocysteine (HCY) concentration in the plasma has been evaluated by HPLC.

Table 1.

The frequency of aspirin and clopidogrel resistance (platelet aggregation)			*Hyperhomocysteinemia HCY>16 µM -19 (38%)*			*Polymorphism C807T GPIa*
ADP 3.5µM	*ADP 5µM*	*Collagen 2µg/mL*	*Arachidonic acid 0.6 mM*	*Moderate 16-30µM*	*Medium 30-100µM*	*Heterozygote CT*
5(10%)	9(18%)	3(6%)	2(4%)	18(36%)	1(2%)	30(60%)

There were no statistically significant correlations between antiplatelet drugs resistance and neither the plasma concentration of HCY nor the C807T GPIa polymorphism. *Conclusions*. 1. Platelet aggregation studies reveal resistance to aspirin and clopidogrel therapy in relatively few patients after myocardial infarction. 2. Platelet C807T glycoprotein Ia (GPIa) polymorphism and moderate hyperhomocysteinemia are very common in survivors of myocardial infarction. 3. There is no interrelationship between resistance to aspirin and clopidogrel therapy and platelet C807T glycoprotein Ia polymorphism or homocysteine plasma concentration.

0910

NUMBER AND MIGRATORY ACTIVITY OF CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN PATIENTS WITH VENOUS THROMBOEMBOLISM

M. Korzh, S.V. Krasnokutskiy, G.I. Kotchuev

Kharkov Medical Academy, Kharkov, Ukraine

Backgrounds. Endothelial progenitor cells (EPC) derived from bone marrow are believed to support the integrity of the vascular endothelium. The number and function of EPC correlate inversely with cardiovascular risk factors, but the prognostic value associated with circulating endothelial progenitor cells has not been defined. Aims. We hypothesized that altered EPC biology may contribute to the pathophysiology of venous thromboembolism (VTE). Methods. EPCs were determined in patients with VTE (n=52) and in a normal control group (n=47) by fluorescence-activated cell-sorting (FACS) analysis. Cells that were positive by flow cytometry for CD34/KDR/AC133 within the lymphocyte population were characterized as EPCs. Results. Patients with VTE showed markedly decreased numbers of EPC (43.7%) and colonies (74.5%) when compared with the controls (p<0.001). These findings were corroborated by 29.8% decrease in EPC migratory function in response to vascular endothelial growth factor (VEGF) (p=0.039) and 47.6% decrease in EPC incorporation into human umbilical vein endothelial cells (HUVEC) (p<0.001). In addition, Framingham's risk factor score of both HIV-infected patients (r=-0.472, p=0.01) and normal group (r=-0.376, p=0.012) significantly correlated with the numbers of EPC. Indeed, the number of circulating EPC was significantly lower in patients with VTE than in normal group under the same burden of risk factors (p<0.001). Conclusions. EPC biology, which is critical for neovascularization and the maintenance of vascular function, is altered in patients with VTE. Our data strongly suggest that dysfunction of circulating EPC has a role in the progression of cardiovascular complications in these patients.

0911

SIGNIFICANCE OF PLASMA THROMBIN-ACTIVATABLE FIBRINOLYSIS INHIBITOR LEVELS IN PATIENTS WITH VENOUS THROMBOEMBOLISM

I.V. Korzh, I.F. Fedotova, V.D. Nemtsova

Kharkov Medical University, Kharkov, Ukraine

Backgrounds. Recently, a new potent inhibitor of fibrinolysis, the thrombin-activatable fibrinolysis inhibitor (TAFI), has been isolated from human plasma. The possibility that TAFI also participates in the mechanism of hypofibrinolysis has not been appraised in patients with venous thromboembolism (VTE). Aims. In the present study, we investigated the plasma levels of TAFI and its relation to urinary albumin excretion in patients with VTE with normo- and microalbuminuria. Methods. Forty-seven patients with VTE (29 with normoalbuminuria, 18 with microalbuminuria) and 45 age-matched normal subjects were enrolled in this study. Results. The plasma level of thrombin-antithrombin complex was significantly increased (23.2±2.4 vs. 7.9±1.3 nmol/liter; P < 0.05), whereas the D-dimer/thrombin-antithrombin complex ratio was significantly decreased (14.6±1.2 vs. 25.4 ± 2.7; p< 0.05), showing the occurrence of hypercoagulability and hypofibrinolysis in patients with VTE. The plasma level of TAFI in VTE was significantly elevated, compared with normal subjects (136.1±10.3 vs. 97.4 ± 4.5%; p< 0.05). The plasma level of TAFI in patients with microalbuminuria was significantly higher than the level in those with normoalbuminuria (183.2 ± 23.4 vs. 117.9±11.9%; p< 0.05) or normal subjects (188.6 ± 23.4 vs. 98.7 ± 4.3%; p<0.01). Univariate analysis showed that the plasma TAFI levels are significantly and proportionally correlated with urinary albumin excretion rate (r = 0.53; p< 0.05) and with plasma soluble thrombomodulin level, a marker of endothelial cell damage, in all patients with VTE (r = 0.47; p< 0.05). Conclusions. These data suggest that increased plasma level of TAFI may be involved in the mechanism of vascular endothelial damage in venous thromboembolism.

0912

SOLUBLE P-SELECTIN LEVELS IN DIABETES MELLITUS PATIENTS WITH CORONARY ARTERY DISEASE

S. Aref

Mansoura University, Mansoura, Egypt

Backgrounds. Type 2 (non-insulin-dependent) diabetes is associated with a marked increase in the risk of coronary heart disease. Platelets play a significant role in coronary artery disease. Soluble P-selectin is an index of platelets activation. Aim of the Work: is to assess the soluble P-selectin levels in Coronary artery disease. *Aim*. is to evaluate the levels of sP-selectin in coronary artery disease in ordeder to determine its clinical significance. *Methods.* Soluble P-selectin levels were measured by ELISA in the peripheral blood of 55 diabetic patients with coronary artery disease [21 acute myocardial infarction (AMI), 20 with unstable angina (UA), 14 with stable angina (SU)], 20 patients with diabetes mellitus without coronary artery disease (DM without), and 10 healthy controls. Results :Soluble P-selectin level was significantly higher in patients with AMI (M±SD; 239.3±13.0 ng/mL), than those with UA (141.5± 15.2 ng/mL), SU (92.1±7.67 ng/mL), DM without (89.8±7.1 ng/mL), and healthy control (69.1±4.5 ng/mL) (p<0.001). In patients with US, sP-selectin was found to be significantly elevated as compared to the SU, DM without and control group. sP-selectin was not significantly different in DM without as compared to controls. The sP-selectin levels was correlated to the duration of diabetes mellitus(R= 0.33, p=0.03). Moreover, sP-selectin level was significantly higher in AMI patients with recurrent anginal attack as compared to that in those with single attack(P 0.041).Multivariate analysis revealed that sP-selectin levels at presentation had high adverse influence on coronary artery insult compared to LDL cholesterol level, degree of hypertension. *Conclusion*. Measurement of soluble p 'selectin level may be helpful marker of impending coronary artery insult in diabetic patients.

0913

HR2 HAPLOTYPE IN PATIENTS WITH VENOUS THROMBOEMBOLISM IN LEBANON

R. Mahfouz, Z. Otrock, G. Zaatari, A. Sabbagh, A. Taher, W. Shamseddeen, I. Khalil, Z. Kanaan

American University of Beirut Medical Ct, Beirut, Lebanon

Backgrounds. Venous thromboembolism (VTE) occurs secondary to a number of hereditary and acquired disorders of hemostasis. A recently recognized polymorphism in factor V gene (His1299Arg; named HR2) has been reported to be a possible risk factor for the development of VTE. This polymorphism varies among different ethnic groups in different parts of the world. The significance of HR2 has not yet been tested in VTE patients in Lebanon. *Aims.* The aim of this study is to assess the possible risk of HR2 haplotype in VTE patients in Lebanon. *Methods.* 50 VTE patients (27 males and 23 females) and 125 healthy subjects (72 males and 53 females), all being of Lebanese origin, were examined for HR2. The average ages for the patients and controls were 43.4±20.2 years and 35.4±18.6 years, respectively. The DNA was extracted using the PEL-FREEZ extraction kit (PEL-FREEZ, DYNAL, USA) and stored at -80°C for later use. The CVD StripAssay (ViennaLab, Austria) was used and its protocol was followed exactly as stated by the manufacturer. This assay screens for several gene mutations including factor V H1299R (HR2). Briefly, *in vitro*, the different gene sequences are simultaneously amplified and biotin-labeled in a single amplification reaction (Multiplexing). The thermocycler program consists of an initial step of 94°C for 2 minutes, followed by 35 cycles of 94°C for 15 seconds, 58°C for 30 seconds, 72°C for 30 seconds, and a final extension step of 72°C for 3 minutes. Finally, the amplification products are selectively hybridized to a test strip which contains allele-specific (Wild type and Mutant) oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and color substrates. *Results.* Data showed that 11 patients and 13 healthy subjects had HR2 haplotype (all were heterozygous for the mutation), with a prevalence of 22.4% and 10.4%, respectively (p = 0.04). 36 patients (72%) had deep venous thrombosis (DVT), 7 patients (14%) had pulmonary embolism (PE), and 7 patients (14%) had both DVT and PE. Furthermore, among the patients who had the HR2 haplotype, 2 patients had the Factor V G1691A mutation, 2 were homozygous for the MTHFR C677T mutation, and none had the prothrombin G20210A mutation. *Conclusions.* In Lebanon, the prevalence of HR2 is significantly high among patients with VTE with an allelic frequency of 0.11. This haplotype has a 2.4-fold greater risk of developing VTE. Moreover, it may coexist with other thrombophilia genetic mutations. Further larger studies are needed to be conducted in the Lebanese population in order to assess whether it is recommended to screen patients with VTE for the HR2 haplotype.

0914

PLATELET GLYCOPROTEIN IA C807T POLYORPHISM AS A RISK FACTOR FOR CORONARY ARTERY DISEASE: A META-ANALYSIS

E. Tsantes,[1] K. Nikolopoulos,[2] G. Bagos,[3] G. Gialeraki,[1] P. Mantzios[1], S. Travlou[1]

[1]*Attikon Hospital, School of Medicine, Athens, Greece;* [2]*Hellenic Center for Disease Control, Athens, Greece;* [3]*Dept. of Cell Biol.& Bioph. Univ. Athens, Athens, Greece*

Background-Aims. Platelet plays a crucial role in the pathogenesis of arterial occlusive disorders and platelet-dependent thromboembolism is considered an underlying mechanism in the pathogenesis of Coronary Artery Disease (CAD). The glycoprotein (GP) Ia/IIa, also known as integrin α2β1, is an important mediator of the adhesion of platelets to fibrillar collagen. Several case-control studies have investigated the importance of the α2 gene (GPIa) C807T polymorphism, a genetic marker of integrin α2β1 surface levels, as a risk factor for CAD, but the research findings were controversial. CAD continues to be the major cause of morbidity and mortality in the Western world. Hence, we carried out a meta-analysis in order to determine the importance of this polymorphism as a risk factor for CAD. *Methods.* Nineteen studies with data on the contribution of GPIa C807T polymorphism to coronary risk were identified through a comprehensive MEDLINE search up until October 2005. We used random effects models to analyse data on studies that specifically examined cases with CAD including Myocardial Infarction (MI) and patients with only MI [including those with Acute Coronary Syndrome (ACS)]. *Results.* The C versus the T allele contrast in the CAD group yielded an OR of 0.998 (95% CI: 0.937-1.064). The combined estimate was also insignificant when we performed the analysis in studies involving cases either with MI or with an ACS (OR, 1.013; 95% CI:0.942-1.089). Similarly, comparing the C with the T homozygotes in the CAD group, we derived a non significant OR (OR, 1.054;95% CI:0.898-1.236) and all other comparisons (CC genotype versus the others or TT genotype versus the rest) did not suggest any gene-disease association. There was no between studies heterogeneity and publication bias might have not influenced the magnitude of the effect. The results remained unaffected when we fitted meta-regression models including variables such as age, risk level, gender, geographical origin, and smoking habits. *Conclusions.* We failed to show that the C807T polymorphism of the β2 gene could influence susceptibility to CAD either as an independent factor or in combination with any conventional risk factor.

0915

CORRELATION OF PLATELET GLYCOPROTEIN IA C807T POLYMORPHISM AND RISK FOR CEREBROVASCULAR DISEASE: A META-ANALYSIS

E. Tsantes,[1] G. Bagos,[2] K. Nikolopoulos,[3] I. Merkouri,[1] S. Travlou[1]

[1]*Attikon Hospital, School of Medicine, Athens, Greece;* [2]*Dept. of Cell Biol.& Bioph. Univ. Athens, Athens, Greece;* [3]*Hellenic Center for Disease Control, Athens, Greece*

Background- Aims. Platelets are crucial in primary haemostasis and their adhesion to damaged vessel wall is mainly mediated by the collagen receptor glycoprotein (GP) Ia/IIa, known as integrin α2β1. Besides limiting blood loss at sites of tissue trauma, platelet thrombi are also responsible for the obstruction of diseased vessels, resulting in ischemia and infarction of vital organs. Although the C807T single nucleotide polymorphism of integrin α 2 gene (GPIa) correlates with increased platelet surface levels of the integrin α2β1, the results concerning the association of this genetic variant with ischemic stroke have been controversial. In order to clarify this association, we performed a meta-analysis of published data regarding this issue. *Methods.* Seven studies providing data on the contribution of GPIa C807T polymorphism to the development of ischemic stroke were found through PubMed search. For the analysis of data, we used random effects models and meta-regression. *Results.* The pooled frequency of the T allele was 36.33% in cases and 37.01% in controls, while the T versus the C allele contrast gave an OR of 1.11 with a 95% CI 0.827-1.499. Furthermore, comparing the T homozygotes with the C homozygotes, we derived a non-significant OR (OR, 1.36; 95% CI: 0.637-2.887). Similarly, the two other contrasts (CC genotype versus the others or TT genotype versus the rest) provided absolutely no evidence of any gene-disease association There was significant between study heterogeneity (p<0.05). In one of the contrasts, the difference in males' percent between cases and controls was significant in the meta-regression suggesting an improper matching with regard to sex. *Conclusions.* This meta-analysis failed to show any significant influence of the 807T allele on the risk of stroke neither in the group of patients as a whole nor in any relevant subgroup. However, due to the significant diversity between a small number of studies in the present meta-analysis, the interpretation of the summary effect has to be done with caution.

0916

A NOVEL HIGH SHEAR RATE ARTERIAL THROMBOSIS MODEL IN BABOONS

P.N. Badenhorst,[1] S. Lamprecht,[1] J.P. Roodt,[2] S.M. Meiring,[2] K. Silence[3]

[1]*University of the Free State, Bloemfontein, South Africa;* [2]*NHLS and University of the Free State, Bloemfontein, South Africa;* [3]*Ablynx NV, Swijnaarde, Belgium*

Background. Animal arterial thrombosis models plays an important part in the evaluation of antithrombotic agents. Two models have been used extensively in this regard, namely those of Hanson et al and Folts et al. Unfortunately both models have limitations such as the utilisation of artificial surfaces and low shear rates, or is technically challenging. We combined elements of both models in a new high shear rate model in baboons. *Aim.* The development of a high shear rate arterial thrombosis model in baboons for the evaluation of anti-platelet agents. The model should allow for platelet thrombi to be formed on an injured stenosed femoral artery. *Methods*. An arterio-venous silicon shunt was established between the femoral artery and the femoral vein. The shunt is non-thrombogenic and allows a 3-5 fold higher shear rate. A flow probe is fitted to the tubing and the femoral artery injured by applying two overlapping occlusions of the artery for one second each using a forceps. A clamp is then placed over the injured site and adjusted to produce an external stenosis of 80% of the flow rate. The injury results in the accumulation of platelets on the injured site which eventually leads to total occlusion. When the vessel is occluded, the clamp is released and the process repeated. This repetitive pattern of decreasing flow is referred to as cyclic flow reductions (CRFs). After 30 min of control CFRs, the test agents: Saline (n=2), Abciximab (n=3), Aspirin (n=3), Clopidogrel (n=4) or Heparin (n=3) were administered at different concentrations and monitored for up to 30 min. This procedure was repeated several times with escalating doses of each test substance. When full inhibition of CFRs was achieved, a new injury was induced to ensure that the inhibition was an effect of the treatment and not a natural healing phenomenon. At the end of the experiments, epinephrin was injected in order to distinguish between a weak and strong inhibition of CRFs. *Results*. No effect on the duration of CRFs was seen with saline, heparin and aspirin. Effective inhibition of CRFs was seen in one animal at a dose of 2.5 mg/kg clopidogrel and in 4/4 animals at doses of 5, 10 and 20 mg/kg. The effective inhibition was, however, readily reversed by infusion of 2.2 μg/kg/min epinephrin. Effective inhibition of CRFs was seen in 2/3 animals at a dose of 100 μg/kg abciximab and in 3/3 animals at doses of 250 and 500 μg/kg. The inhibition could not be reversed by infusion of 2.2 μg/kg/min epinephrin. *Conclusion*. This model is suitable for the evaluation of anti-platelet agents in baboons.

0917

B-THROMBOGLOBULIN IN CHILDREN WITH POSITIVE FAMILY HISTORY OF CORONARY ARTERY DISEASE

N. Lefkou, E. Ioannidou, S. Vakalopoulou, V. Perifanis, S. Theodoridou, V. Garipidou, M. Athanasiou, I. Tsiouris, I. Klonizakis

Ippokrateion University Hospital, Thessaloniki, Greece

Introduction: Platelets are recognized to play a key role not only in thrombus formation in acute coronary syndromes, but also, as inflammatory cells, at the onset and progression of atherosclerosis process. *Aim*: To investigate whether platelets are activated in children with positive family history of coronary artery disease (CAD), contributing consequently to a premature initiation of atherosclerotic process. *Methods.* We studied 55 healthy children (5-15 years), 30 (16 male) with family history of CAD (group A) and 25 without positive family history (12 male) (group B) who were used as a control group. B-thromboglobulin (b-TG), as a marker of platelets activation, was measured from platelets taken from peripheral blood sample in all individuals included in the study. Glucose, white blood count (WBC), platelets, total cholesterol, triglycerides (TG), HDL, LDL, sedimentation rate (SDE), CRP, PAI-1 and t-PA were also measured from blood sample. *Results.* Children in group A had statistically significantly higher values of b-TG compared to children of Group B (72,95±15,4 vs 42,65±9,07 ng/mL, p<0,05). Among children with positive family history of CAD boys had higher values compared

to girls. A strong relationship between b-TG, atheromatous index, PAI-1 and triglycerides was also found in children with positive family history of CAD. *Conclusion*: Platelets activation takes place during childhood in children with family history of CAD. Those findings suggest that the inflammatory process of atherosclerosis begins early in childhood in individuals whose parents have developed prematurely coronary artery disease.

0918

SERUM LEVELS OF SOLUBLE E-SELECTIN IN VENOUS THROMBOEMBOLISM (VTE)

J. Gonzalez-Ordonez,[1] E. Gonzalez,[2] C. Fernandez,[2] M.D. Macias,[1] M.A. Arias,[1] M. Peliz,[1] M. Moran[1]

[1]*Hospital San Agustin, Aviles, Spain;* [2]*Hospital de Cabuees, Gijon, Spain*

Background. The inflammatory response of the vein endothelium seems to have relevance in the acute phase of the venous thromboembolism (VTE) and involves a strong expression of some cell adhesion molecules (CAMs). The serum levels of the soluble form of the E-selectin (sE-selectin) are related with the activation of endothelial cells but the evolution of their values in a later (chronic) phase of the VTE is not known. We aim to identify any association between the sE-selectin and the VTE six months after the acute phase. *Population and Methods*. We measured the serum sE-selectin concentrations from 394 subjects: 197 consecutive patients objectively diagnosed of venous thromboembolism (VTE) six months after the acute phase and 197 controls of similar gender and age [61.5(12.9) years, 49.0% males] by an enzyme-linked immunosorbent assay (ELISA) method. The non-Gaussian distribution of the sE-selectin values requires the use of non-parametric statistical tests. *Results.* In the overall series, the level of the sE-selectin directly correlates with the waist /hip ratio (r= 0.21, $p<0.0001$) being higher among the males [66.0(54.5) vs. 55.5(38.1) ng/mL, $p=0.001$] although showing a weak inverse correlation with the age (r= -0.135, $p<0.01$). The sE-selectin was independent of the body mass index (NS p). A trend to lower sE-selectin values appears among the patients [56.5(43.6) ng/ml] versus the controls [65.0(47.6) ng/ml] ($p=0.055$, Mann Whitney test). However the extreme values did not show association with the VTE [90th percentile (124.5 ng/mL): OR= 1.06, NS p and 10th percentile (33.1 ng/ml): OR= 1.17, NS p]. The soluble E-selectin was also similar in recurrent (n=50) and non-recurrent cases [55.3(33.4) vs. 57.0(45.2) ng/ml] (NS p). *Conclusion*. The soluble E-selectin values were not clearly related with the VTE in a late phase (six months after the acute episode).

Funding: This study was supported by the grant FIS #04/1550.

0919

NO EFFECT OF B-VITAMIN SUPPLEMENTATION ON MARKERS OF THROMBIN GENERATION IN PATIENTS WITH VENOUS THROMBOEMBOLISM

C.A. Rodrigues,[1] R.C. da Silveira,[1] M.A.E. Noguti,[1] V.M. Morelli[1], V. D'Almeida,[1] A.A. Garcia,[2] F.H.A. Maffei,[3] D.M. Lourenco[1]

[1]*Universidade Federal de Sao Paulo, Sao Paulo, Brazil,* [2]*Universidade de Sao Paulo, Ribeirao Preto-SP, Brazil,* [3]*Universidade Estadual Paulista, Botucatu-SP, Brazil*

Backgrounds. Mild hyperhomocysteinemia is associated with an increased risk of venous thromboembolism (VTE) and other cardiovascular diseases. *In vitro* studies showed that homocysteine may stimulate procoagulant factors and increase thrombin generation. Folic acid and B-vitamins supplementation decrease homocysteine levels, but it is not clear whether this may interfere with its procoagulant effects. *Aims.* To evaluate the effect of vitamin supplementation on the homocysteine level and on markers of thrombin generation in patients with VTE. *Methods.* This study was a multicentre, randomized, double-blind, placebo-controlled trial. We randomized 105 patients with a first event of objectively confirmed VTE, aged between 18 and 70 years, to receive either vitamin supplementation (folic acid 5 mg, vitamin B6 50 mg and vitamin B12 0.4 mg) or placebo. Blood was collected at randomization and 8 weeks after the intervention period. *Results.* Ninety-nine (94.3%) completed the 8-week period of treatment. There was no difference between patients with homocysteine above the highest tertile (12.6 micromol/L) and those below the lowest tertile (9.9 micromol/L) in the levels of prothrombin fragment 1+2 (median 0.73 and 0.71 nmol/L, respectively) , thrombin-antithrombin complex (median 4.5 and 4.1 microg/L) and D-dimer (median 277 and 256 ng/mL). In patients treated with vitamins, there was a 29% decrease in the homocysteine levels. However, prothrombin fragment 1+2, TAT and DD levels did not change, both in the vitamin and in the placebo groups. Besides, treatment with vitamins had no effect on these markers, even in patients above the highest tertile of homocysteine. *Conclusions.* In patients with VTE, higher homocysteine levels are not associated with higher levels of thrombin generation markers and homocysteine reduction by B-vitamin supplementation had no effect on these markers.

0920

IMPACT OF A DIAGNOSTIC PROTOCOL FOR DEEP VEIN THROMBOSIS ON REQUESTS FOR D-DIMER ASSAYS

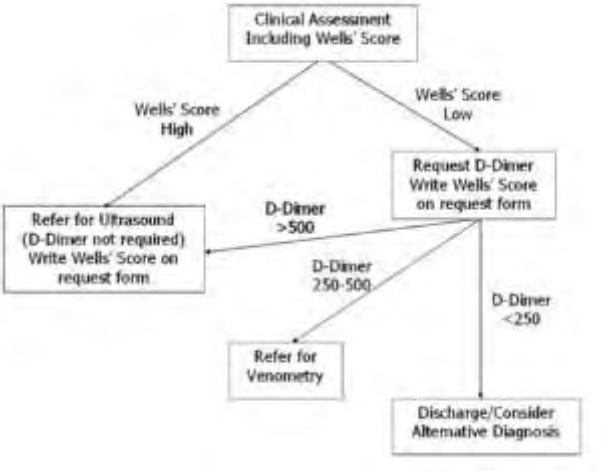
J. Bowers, Y.L. Ong, R. McLaughlin

Ulster Hospital, BELFAST, United Kingdom

Backgrounds. Sensitive D-dimer assays have been shown to be useful for decreasing the need for formal radiological imaging in the diagnosis of Deep Vein Thrombosis (DVT) in selected patients who have low clinical pretest probability (PTP) scores.[1] Aims. Unfortunately in our institution the PTP score was not always assessed and the D-dimer test was therefore being used to rule out DVT even in those patients who would have had a high PTP. This is inappropriate and has resulted in missed diagnoses. Methods. In Jan 2005 we introduced a diagnostic protocol in A+E, which relied on a Wells' PTP Score (Figure 1). Patients with low PTP (0 or 1) and D-dimer value < 250 ng/L did not require radiological imaging and were discharged. The haematology laboratory was empowered to reject samples for D-dimer testing if the request form did not contain a Wells' PTP. In contrast, patients with a high PTP (2 or 3) proceed to radiological imaging without a D-dimer test. An audit of compliance with the new pathway was assessed, and an action plan formulated to promote awareness and compliance. Results. The results were compared one year later: the number of D-dimer requests has decreased by 50% with the new protocol. There are now no apparent missed DVTs (as indicated by patients who returned to the A+E department and later were confirmed to have DVTs in the year prior to introduction of the protocol). Summary: D-dimer assays must be used in conjunction with a clinical pre-test probability score; a low PTP and a negative D-dimer can reliably exclude DVT. Prior to the introduction of this protocol we received 350 D-dimer requests per month. The introduction of a Wells' PTP and selective use of D-dimer testing resulted in cost savings, BMS time efficiency and significant relief in manpower pressures in providing the 24-hour D-dimer service.

References

1. BCSH. BJHaem, 124,15-25



0921

RELATIONSHIP BETWEEN HOMOCYSTEINE LEVELS AND MTHFR GENOTYPE, AND THEIR EFFECT ON DEEP VENOUS THROMBOSIS

L. Pantelidou,[1] A. Agorasti,[2] I. Bazdiara,[1] G. Trypsianis[1], D. Margaritis,[1] E. Spanoudakis,[1] M. Baka,[1] S. Kotsiopoulou[1], I. Kotsianidis,[1] D. Konstantinidou,[2] G. Bourikas[1]

[1]*Democritus University of Thrace, ALEXANDROUPOLIS, Greece;* [2]*General Hospital of Xanthi, XANTHI, Greece*

Backgrounds. High serum total homocysteine concentrations (tHcy) are suggested to be a risk factor for arterial and deep venous thrombosis. 5,10-methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme in homocysteine metabolism. The mutation MTHFR C677T renders the enzyme thermolabile and leads to elevated tHcy levels. *Aim*: In this study the relationship between tHcy levels and MTHFR C677T polymorphism in patients with first episode of deep venous thrombosis (DVT), as well as the assessment of hyperhomocysteinemia as risk factor for DVT were investigated. *Methods.* 46 (20 men, 26 women) healthy individuals (group A) aged 41.59±14.11 years and 74 (37 men, 37 women) patients with first episode DVT (group B) aged 45.99±14.98 years were enrolled. Total homocysteine levels were determined with ACS: 180' SE Automated Chemiluminescence Systems (Bayer). MTHFR genotypes were analyzed using PCR amplification and digestion with restriction endonuclease Hinf I. The data were expressed as the mean ± SD and analyzed with Student test, Univariate Analysis of Variance, Tukey test, Logistic Regression Analysis. Values of $p<0.05$ were considered to indicate statistical significance. Hyperhomocysteinemia was set at 90th percentile of tHcy levels of group A. *Results.* Statistically significant difference in tHcy levels between groups A and B was observed (A vs B, 12.44±4.43 vs 14.54±5.37 μmol/l, $p=0.029$). The frequency of alleles was 0.598/0.689 for C allele and 0.402/0.311 for T allele in groups A and B, respectively. Among the subjects with T/T genotype, higher tHcy concentrations were detected in group B than group A (T/T, A vs B, 13.07±4.67 vs 22.47±7.22, $p=0.012$). No important difference was found in the tHcy levels between the two groups with respect to C/C ($p=0.141$) and C/T ($p=0.292$) genotype (A vs B, C/C: 11.28±4.17 vs 13.07±3.96, C/T: 13.14±4.55 vs 14.60±5.16). There was no effect of MTHFR C677T mutant genotype on tHcy levels in group A. Total Hcy concentrations in patients (group B) with T/T genotype are statistically higher when compared to C/C (C/C vs T/T, 13.07±3.96 vs 22.47±7.22, $p<0.001$) and to C/T (C/T vs T/T, 14.60±5.16 vs 22.47±7.22, $p=0.001$) genotypes. Hyperhomocysteinemia (tHcy > 19.30 μmol/l) was observed at 8.7% (4/46) of group A and 21.6% (16/74) of group B. Logistic regression analysis indicated that only hyperhomocysteinemia is an independent risk factor for DVT (Odds Ratio=3.95, CI 95%: 1.1-14.4, $p=0.037$), while the genotype ($p=0.268$) and the interaction between genotype and hyperhomocysteinemia ($p=0.568$) are not risk factors. Conclusions. Our results indicated that patients with T/T genotype have higher tHcy levels when compared to healthy individuals as well as to patients with C/C and C/T genotypes. Hyperhomocysteinemia is an independent risk factor for deep venous thrombosis. The T/T genotype and the combination of hyprehomocysteinemia and T/T genotype are not related to deep venous thrombosis.

Platelets/Thrombocytopenia

0922

ADAMTS-13 GENE MUTATION IN A PATIENT WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP)

A. Falanga,[1] M. Marchetti,[1] M. Noris,[2] S. Nosari,[2] L. Russo[1], T. Barbui,[1] G. Remuzzi[3]

[1]*Hematology Div. Ospedali Riuniti, Bergamo, Italy;* [2]*Mario Negri Institute Pharmacol Res, Bergamo, Italy;* [3]*Mario Negri Inst and Ospedali Riuniti, Bergamo, Italy*

Background. The vWF-cleaving protease (ADAMTS-13) is important for maintaining the normal size distribution of vWF multimers. A severe deficiency of ADAMTS-13 activity (i.e. <5% of that of normal plasma), caused by either mutation of the ADAMTS-13 gene or by inhibitory auto-antibodies to ADAMTS-13, is associated to TTP. *Aim and Methods*. We describe the case of a 19-year-long chronic relapsing TTP. A 45-year woman was first diagnosed with TTP, during pregnancy, in 1987 when she was 27 years. Subsequently, she relapsed three times in 1993, once in 1996, and once in 2000. On each episodes she was treated with plasma exchange and steroid therapy, obtaining the complete remission. *Results.* In 2001, the ADAMTS-13 testing became available in our laboratory and we prospectively followed the patient for ADAMTS-13 activity and inhibitors. In addition, since 2005, we also tested the plasma levels of anti-ADAMTS-13 antibody. In 2004 she relapsed after starting interferon for HCV-related chronic hepatitis. Plasma exchange and steroid therapy were resumed, without achieving a durable remission, as she relapsed after one month. She was then given chemotherapy (i.e. fludarabine, cyclofosfamide) and rituximab, without significant response. From January 2005 on, she is receiving periodic plasma infusion on the basis of platelet count and is continuing on this regimen so far. The measurement of ADAMTS-13 activity from 2001 showed a severe deficiency (<5%) of this protease during clinical remissions and upon relapses. No significant plasma inhibitory activity was found by mixing studies. The retrospective quantification of auto-antibodies by ELISA revealed no significant levels of anti-ADAMTS-13 antibodies. The patient was then identified as a possible carrier of a true constitutive ADAMTS-13 deficiency. The DNA analysis of this patient detected homozygosity for the 3428 C>T in exon 25 of the ADAMTS-13 gene, which predicts the R1123C exchange in the TSP1-8 domain. The inherited nature of severe ADAMTS-13 deficiency was established by family analysis. *Conclusions*. This mutation has been previously linked to Upshaw-Schulman syndrome (USS), a congenital chronic relapsing form of TTP, characterized by neonatal onset, response to fresh plasma infusion, and frequent relapses. Differently, in our patient the onset of clinically overt disease manifested in the adult age during pregnancy, thus supporting the hypothesis that additional precipitating factors may determine the phenotypic manifestation of this mutation.

0923

IS THE VARIABLE CLINICAL PRESENTATION IN HEREDITARY TTP THE RESULT OF DIFFERENCES IN RESIDUAL ADAMTS13 ACTIVITY?

R. Zahnd,[1] B. Lämmle,[2] J.A. Kremer Hovinga[1]

[1]*Central Hematology Laboratory, Bern, Switzerland;* [2]*Inselspital, University Hospital Bern, Bern, Switzerland*

Backgrounds. Hereditary TTP (Upshaw Schulman syndrome, USS) is the consequence of severe ADAMTS13 deficiency (<5% of the normal) due to homozygous or compound heterozygous ADAMTS13 gene mutations. Analysis of patient histories revealed a striking age-dependent clustering of the first TTP attack. While half of the patients develop clinical signs of acute TTP immediately after birth or in early childhood (early onset), the other half remains asymptomatic into early adulthood and suffers from a first acute TTP episode at the age of 20-40 years (late onset) and this clinical pattern is often very similar in affected siblings. So far, no correlation between the clinical phenotype, i.e. disease severity, organ tropism and the underlying genotype is discernable. In analogy to the situation in hemophiliacs (distinction between <1% and 1-5%) determination of residual ADAMTS13 activity could help to elucidate the variable clinical presentation. As VWF levels increase with age a critical threshold might eventually be exceeded so that these patients' residual ADAMTS13 activity is no longer sufficient to prevent TTP bouts. *Methods.* Index patients from 28 families with USS were included into this study. Only plasma samples withdrawn at least 4 weeks

after the last plasma infusion were analyzed. ADAMTS13 activity was determined by a new fluorescence resonance energy transfer assay using a synthetic, truncated 73-amino-acid VWF peptide as a substrate (FRETS-VWF73 assay; Kokame et al. Br J Haematol. 2005;129:93-100), which was modified in order to reliably distinguish between 0% and 1% of ADAMTS13 activity, which had not been possible with the older assays. *Results.* Half of the USS patients (14/28) had an ADAMTS13 activity <1% by FRETS-VWF73; 11 patients displayed a residual activity between 1-5% and in three instances ADAMTS13 activities lay between 5 - 8%. Attempts to link patients histories with their ADAMTS13 activities failed, as patients from both clinical groups (early vs. late onset) had ADAMTS13 activity values <1% or in the range of 1-5%. *Conclusions.* Differences in residual ADAMTS13 activity are apparently not accountable for the documented age-related presentation in USS. It is thus likely, that other disease-modifying genetic (i.e. blood group, VWF levels) or environmental factors affect the phenotype.

0924

INCIDENCE AND LABORATORY FEATURES OF THROMBOCYTOPENIA IN 43 PATIENTS WITH VON WILLEBRAND DISEASE TYPE 2B: CORRELATION WITH MOLECULAR DEFECTS AND ACQUIRED MODIFICATIONS OF VWF

A.B. Federici,[1] L. Baronciani,[3] M.T. Canciani,[4] B. Moroni,[4] F. Gianniello,[4] A. Artoni,[4] C. Balduini,[5] P.M. Mannucci[4]

[1]*University of Milan, Milan, Italy;* [2]*A. B. Bonomi Hemophilia Thrombosis Ctr, Milan, Italy;* [3]*A.B.Bonomi Hemophilia Thrombosis Ctr, Milan, Italy;* [4]*Department of Internal Medicine, Milan, Italy;* [5]*University of Pavia, Pavia, Italy*

Backgrounds. Von Willebrand type 2B (VWD) is an inherited bleeding disorder caused by abnormal von Willebrand factor (VWF) that displays increased affinity to the platelet glicoprotein 1b α (GpIba). VWD 2B is due to a group of mutations clustered within VWF A1 domain and is characterized by binding of its high molecular weight multimers (HMW) to platelets often resulting in moderate-mild thrombocytopenia. Even though there are many case reports on thrombocytopenia associated with VWD 2B, retrospective and prospective studies in a large cohort of patients are not available. *Aims and design of the study*. to determine incidence and laboratory features of thrombocytopenia in VWD 2B, we have prospectively observed our cohort of 43 patients (18 families) previously characterized by VWF mutations. Methods. Data of platelet count with mean platelet volume (MPV) and morphologic evaluation of the blood smear to search for giant platelets or aggregates were associated with the history of physiologic or pathologic stress conditions such as pregnancy, infections, surgery or use of DDAVP. All patients were characterized by ristocetin induced platelet agglutination (RIPA) in the Platelet Rich Plasma (PRP), ristocetin cofactor activity (VWF:RCo) with VWF antigen (VWF:Ag), multimeric structure of VWF. Mutations within VWF A1 domain were searched for and confirmed by sequencing exon 28.

Table 1.

			Low plt. (<140×10⁹)		Plt.morphology	
Mutation (n)	RIPA (mg/mL)	VWF:Ag (U/dL)	Basal (n)	Post stress (n)	MPV (μm3)	gp/aggr.
R1306W (15)	0.65	27	4	15	10.3	3
R1308C (5)	0.72	40	2	5	11.5	2
R1308L (5)	0.50	37	0	0	9.1	0
I1309V (6)	0.40	79	2	6	11.8	0
V1316H (3)	0.50	45	2	3	9.2	1
P1337L (4)	0.50	39	0	4	9.5	0
P1341Q (4)	0.67	43	0	0	9.9	0
R1341W (1)	0.70	43	1	1	9.9	0

Results. Among 43 VWD cases, a platelet count< 140,000 was found at baseline in only 11 (26%), but was observed after stress conditions in 34 cases (79%); no reduced platelet counts was found in 9 patients (21%) from two different families (R1308L, R1341Q).An increased MPV was found in 35 cases but giant platelet and aggregates in only 6 cases. All the phenotypic features were correlated to VWF mutations. *Conclusions.* Based on these results, thrombocytopenia can be associated in most VWD 2B patients, especially when high levels of mutant VWF are triggered by physiologic and pathologic stress conditions. However, not all VWD 2B show thrombocytopenia and a relatively high degree of heterogeneity of this phenomenon occurs within patients characterized by the same molecular defects.

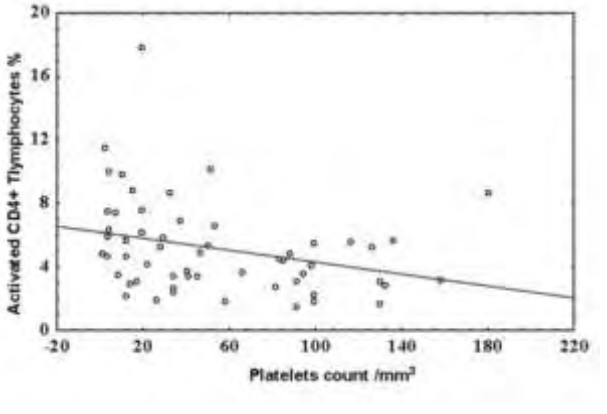
0925

DIFFERENT ACTIVATION STATUS IN PERIPHERAL VERSUS SPLENIC T LYMPHOCYTES IN IMMUNE THROMBOCYTOPENIC PURPURA PATIENTS

M.S. Dilhuydy, P. Blanco, A. Gomez, J.F. Moreau, J.L. Pellegrin, J.F. Viallard

CHU Bordeaux, Bordeaux, France

Backgrounds. Adult idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by enhanced splenic destruction of the platelets, through autoantibodies binding to membrane glycoproteins. B lymphocytes secreting antiplatelet antibodies are considered as the major mecanism in ITP. Nevertheless, cellular immunity could have a role in ITP pathophysiology. Recently, autoreactive $CD4^+$ T cells directed against membrane platelets proteins have been identified and another group pinpointed the implication of $CD8^+$ cytotoxic T lymphocytes for a direct cell-mediated lysis of autologous platelets in active ITP. *Aim*: We explored activation status of both $CD8^+$ and $CD4^+$ T-lymphocyte subsets in chronic ITP patients in peripheral blood and spleen when splenectomy was indicated. We surmised that different T-lymphocyte activation status could reflect different pathogenic mechanisms involved in platelet destruction. *Methods.* Fifty four patients with chronic ITP were enrolled prospectively in the present study and compared to 46 normal healthy volunteers. Among ITP group 17 patients had a splenectomy. Phenotypical analysis was done on ITP splenic T-lymphocytes and compared to T-lymphocytes obtained from post traumatic splenectomy. Flow cytometry was used to evaluate T-lymphocyte HLA-DR membrane expression. We used Wilcoxon-Mann-Whitney test to compare continuous variables, as they did not present normal distributions. We performed a Bonferronni adjustment to prevent the raise type I errors due to multiple testing between groups. *Results.* All 46 patients fulfilled ASH criteria for chronic ITP. Their ages ranged from 16 to 79 years with a median age of 49 years. Sixteen patients were male and 38 female. Median platelet count was 42500/mm³ (1000-136000/mm³). The percentage of $CD3^+DR^+$ peripheral T-lymphocytes was significantly higher in ITP patients (10.79% vs 7.20% p=000.4), with predominance for activated $CD4^+$ subsets (6.12 vs 2.71 p<0.0001) compared to actived $CD8^+$ (7.6 vs 3.36 p=0.0045). This activation was correlated with platelet counts for both subsets ($p<10^{-6}$) (Figure 1).



Nevertheless, this activation status was not correlated to treatment efficacy nor prognosis. Study of splenic T-cell subset activation reveals different results. Indeed, only $CD3^+CD8^+$ splenic lymphocytes were found activated in ITP spleen, compared to controls (14.53% [4.96 - 38.65] vs 7.12%[2.55 - 13.27] p=0.008). Conclusion: From the present study we can conclude that there is a correlation between the severity of ITP and the increased percentage of activated T lymphocytes. Nevertheless, there is no correlation between this activation and prognosis. Interestingly, refractory ITP are characterized by an increase of splenic activated $CD8^+$ that could be involved in platelet destruction. This observation may corroborate the possible implication of different pathogenic pathways involved in ITP.

0926

PREVALENCE AND RELEVANCE OF HEPARIN-INDUCED ANTIBODIES IN LMWH-TREATED PREGNANT WOMEN

U. Harbrecht, E. Forrest, C. Gnida, S. Ohlenforst, B. Rott, J. Rox, J. Oldenburg

Institute of Experimental Haematology, Bonn, Germany

Backgrounds. Heparin-induced antibodies (HI-Ab) and heparin-induced thrombocytopenia (HIT) have been demonstrated less frequent due to LMWH than UFH, however, this has been questioned in non-surgical patients more recently. So far no HIT cases in LMWH-treated pregnant women have been reported, whereas thrombocytopenia of other aetiology may develop during pregnancy. Aims. The purpose of this study was to investigate whether women treated with LMWH during pregnancy present with or develop HI-Ab and whether HI-Ab are of clinical relevance. *Patients and Methods.* 111 women with a history of thrombosis (n=71), risk factors for thrombosis (n=40) and/or recurrent fetal loss (n=19) completed 121 pregnancies and were treated with LMWH or danaparoid (nadroparin n=101, dalteparin n=9, enoxaparin n=9, certoparin n=6, danaparoid n=5) during pregnancy and for 4 to 6 weeks after delivery. Inherited thrombophilia was diagnosed in 68/111 (61.3%) patients, 40 of them (59%) had experienced thrombosis. LMWH was initiated between week one and 30 of pregnancy depending on the defect and thrombosis risk. Development of HI-Ab was investigated by heparin-platelet-factor-4-ELISA (Asserachrom HPIA, Roche, Germany) in 4-8 week intervals. Positive ELISA results were additionally tested by the heparin-induced platelet-activation-assay (HIPAA). Platelet count was monitored once a week during the first 6 weeks of LMWH treatment and thereafter every 4-8 weeks. All measurements of HI-Ab were performed after termination of heparin treatment unless platelet count dropped for more than 50% or thrombocytopenia (< 150 G/l) developed. *Results.* HI-Ab were detected in 6/121 (5.0%) pregnancies by ELISA, none was positive in the HIPAA. Four of the six patients had a history of previous UFH exposure. Four patients had low (OD <1,0) and two intermediate HI-Ab-titres (OD 1.0'2.0; Cut-off-OD 0.43-0.67 depending on the batch). In two patients with low titres (OD 0.63 and 0.56) HI-Ab were already present before LMWH treatment and normalised in one during LMWH administration. Interestingly, two patients with repeated pregnancies revealed HI-Ab during the first but not the second pregnancy. None of the six patients with HI-Ab developed thrombocytopenia or a platelet drop >50%. However, in 8/115 (7.0%) pregnancies without HI-Ab mild thrombocytopenia (range 105-147 G/l) became apparent mostly in the third trimester. Local allergic reactions occurred in 7.2% of patients and required change of anticoagulation. *Conclusions.* Heparin-induced antibodies in pregnants treated with LMWH are detectable with low frequency and usually low titres. Antibodies might in part be due to previous UFH exposure. None of the HI-Ab-positive patients developed thrombocytopenia, hence, the risk of LMWH-induced thrombocytopenia in pregnant women appears to be very low. Most thrombocytopenias are pregnancy-associated and of mild type, however it might be difficult to distinguish between heparin and other factors as cause.

0927

INCREASED PLATELET-MONOCYTE AND PLATELET-NEUTROPHIL COMPLEX FORMATION IN PRIMARY RAYNAUD PHENOMENON AND IN RAYNAUD PHENOMENON SECONDARY TO SYSTEMIC SCLEROSIS

G.E. Pamuk, B. Turgut, O.N. Pamuk, M. Demir, N. Çakir

Trakya University Medical Faculty, Edirne, Turkey

Backgrounds. Although it was suggested that platelet activation in systemic sclerosis (SSc) was secondary to microvascular damage, there is also data that it is the primary event. As there is both platelet and leucocyte activation in both primary raynaud phenomenon (RP) and RP secondary to SSc, it is possible that increased platelet-leucocyte interaction contributes to coagulation system alterations in RP patients. It was stated that platelet-leucocyte interaction was an important factor in the pathogenesis of vascular ischemic syndromes. In addition, active platelets secrete microparticles (PMP) with procoagulant activity. This is the first study to evaluate platelet-monocyte complexes (PMC), platelet-neutrophil complexes (PNC), and PMPs in RP. *Aims.* We evaluated platelet activation markers and PMC, PNC in patients with primary RP and in RP secondary to SSc. *Methods.* We utilized whole blood flow cytometry to quantify the expression of CD62P, PMP, and the percentages of PMC and PNC in primary RP patients and in SSc patients with secondary RP. *Results.* We included 16 consecutive SSc patients with secondary RP (15F, 1M, mean age:44.3), 12 primary RP patients (10F, 2M, mean age:33.6), and 18 healthy subjects (16F, 2M, mean age: 39.2) as our control group. The mean duration of symptoms in primary RP patients was 7.2 years, and it was 8.4 years in patients with secondary RP. CD62P expression in SSc patients with secondary RP was significantly higher than in primary RP patients and in controls (p values, respectively, 0.017 and 0.004). PMC and PNC levels, on the other hand, were significantly higher in both primary and secondary RP groups than in controls (all p values ≤0.001). Although PMP level in primary RP group was higher than in controls, this difference was not significant (p=0.1). (Table 1). PMP level in SSc patients with pulmonary artery hypertension (PAH) was significantly higher when compared to those without PAH (4±0.5 vs. 3.4±0.6, p=0.048). The other parameters evaluated in SSc patients did not significantly differ between groups with or without digital ulcers or loss, those with or without interstitial lung disease, aspirin-users and nonusers (p>0.05). In addition, PMP level had a correlation with pulmonary artery pressure (r=0.59, p=0.017). There was a trend towards higher PMP levels in the anti-centromere-positive group (4.1±0.4 vs. 3.5±0.8, p=0.1). In primary RP patients, PMC level had positive correlations with PNC (r=0.68, p=0.015) and CD62P (r=0.61, p=0.035). In SSc patients with secondary RP, PMC level had a positive correlation with PNC (r=0.88, p<0.001); CD62P level had a negative correlation with PMP (r=-0.5, p=0.046). In 4 patients administered iloprost, the mean CD62P level decreased significantly (16±17.4 vs. 7.4±4, p=0.03); PMC (68±20 vs. 50±10) and PNC levels (34±11 vs. 27.5±6.5) regressed nonsignificantly (p values >0.05). *Conclusion.* Our results suggest that platelet-leucocyte complex formation is increased in RP. In addition, it provides evidence that there is ongoing platelet activation and platelet-leucocyte interaction in SSc patients despite antithrombotic therapy. This result is important to consider as it might have potential therapeutic implications with respect to the use of antiplatelet drugs in these patients.

Table 1. CD62P,PMC,PNC,PMP in RP patients.

	Primary RP	*RP secondary to SSc*	*Controls*
CD62P (%)	6.7±3.8	17.7±13.9	6.1±8.6
PMC (%)	67±20.1	64.1±27.3	22.4±9.1
PNC (%)	37.2±22.8	33.3±16.6	9.2±4.1PMP (%)
4.2±1.4	3.7±0.7	3.5±0.5	

CD62P: SSc is different from in primary RP and controls (p values 0.017 and 0.004); PMC: primary and SSc are different from controls (all p values < 0.001); PNC: primary RP and SSc are different from controls (p values 0.001 and < 0.001).

0928

LOW RATE OF LONG LASTING REMISSIONS AFTER SUCCESSFULL TREATMENT OF IMMUNE THROMBOCYTOPENIA WITH RITUXIMAB

C. Schweizer, J.W. Schmier, A.D. Ho, M. Hensel

University of Heidelberg Medical School, Heidelberg, Germany

Backgrounds. Immune thrombocytopenic purpura is characterised by peripheral platelet destruction due to autoantibodies derived against surface glycoproteins. Management of patients with autoimmune thrombocytopenias is difficult, relapses are common. Recent studies have shown that the anti-CD20 antibody rituximab is effective in the treatment of relapsed and refractory patients. *Aims.* The aim of this study was to evaluate rituximab therapy in ITP patients in our institution. *Methods.* We report the results of a retrospective analysis of rituximab treatment in 14 patients with immune thrombocytopenic purpura. All patients had received 1-7 lines of previous therapies, 4 had undergone previous splenectomy. Rituximab was administered at the standard dose of 375 mg/m^2 once per week with a mean of 4 infusions (range 1-4). *Results.* The overall response rate was 64%. 7 of 14 patients (50%) achieved a complete remission (platelet levels >100×10^9/L). 2 of 14 patients (14%) showed a partial remission (platelets >50×10^9/L). 5 patients did not respond to the therapy. The median time to response after the start of the rituximab treatment was 4 weeks (range 1 - 4). Three patients (21%) had a long lasting and ongoing remission up to 156 weeks. Responding patients remained in remission for a median period of 8 weeks (range 10 days - 36 months). All of the 4 splenectomized patients had a complete remission after rituximab therapy, with 2 long lasting remissions for 26 and 156 weeks. *Summary/Conclusions.* Our observations show that rituximab treatment represents a well tolerated and effective therapy for patients with autoimmune thrombocytopenias even in previously refrac-

tory patients. Nevertheless, there is only a low rate of long lasting remissions. It seems that patients after splenectomy have a higher remission rate and a longer duration of their response.

0929

AUTOIMMUNE THROMBOCYTOPENIA: FLOW CYTOMETRIC DETERMINATION OF PLATELET-ASSOCIATED CD154/CD40L AND CD40 ON PERIPHERAL BLOOD T AND B LYMPHOCYTES

M. Meabed, S. Omar, G. Taha, K. El-Hadidy

Bani-Suef Faculty of Medcine, Bani-Suef, Egypt

Background and Objectives. The CD40-CD40L system has pleiotropic effects in a variety of cells and biological processes including immune response. Within the immune system, these molecules represent a critical link between its humoral and cellular arms. Immune or idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by antibody-induced platelet destruction and clearance because of anti-platelet autoantibodies, which bind to circulating platelets resulting in their destruction by the reticuloendothelial system. Despite its clinical importance, the diagnosis of ITP is one of exclusion, thus, inevitably associated with potential difficulties. CD40 is a cell surface receptor that belongs to the tumor necrosis factor-receptor (TNF-R) family, and that was first identified and functionally characterized on B lymphocytes. CD40-ligand (CD40L/CD154), a member of the TNF superfamily, is a cell membrane molecule expressed on activated CD4+ T lymphocytes and is essential for the T cell-dependent activation of B lymphocytes. Therefore it is now thought that CD40-CD40L interactions play a more important role in ITP immune regulation. Design and Methods. The expressions of CD154 and CD40 on peripheral blood (PB) T and B lymphocytes, respectively, were measured using the technique of flow cytometry. An antigen-specific assay for platelet-associated antibody CD154 (CD40L) on $CD4^+$ T lymphocytes and for CD40 on $CD19^+$ B lymphocytes was tested in 30 children patients with acute ITP, 30 adult patients with chronic ITP, and in 20 age- and sex-matched healthy controls. *Results.* The expressions of $CD4^+CD154^+$ and of $CD4^+CD154^+/CD4^+$ on PB T lymphocytes, and of $CD19^+CD40^+$ and of $CD19^+CD40^+/CD19^+$ on PB B lymphocytes were significantly higher in acute and chronic ITP patients compared to controls, and in acute patients compared to chronics (p<0.001). *Conclusions.* CD40-CD40L interaction plays an important role in the pathology of certain autoimmune diseases. ITP is an autoimmune disease characterized by increased platelet destruction caused by anti-platelet autoantibodies, which mainly target a platelet surface antigen. It is speculated that platelet-associated CD154 is competent to induce the CD40-dependent proliferation of B lymphocytes. Therefore, platelet-associated CD154 expression is increased in ITP patients and is able to drive the activation of autoreactive B lymphocytes in this disease. These findings are particularly useful for clarifying the pathogenic process in ITP patients and for developing a therapeutic approach that blocks pathogenic anti-platelet antibody production. Blockade of the CD40/CD154 signal is a potential immunomodulatory strategy for T-cell-mediated diseases, and many findings suggest that CD40/CD154 blockade therapy is potentially effective for ITP through selective suppression of autoreactive T and B lymphocytes to platelet antigens.

0930

AGONIST INDUCED PLATELET ACTIVATION IN A HEALTHY POPULATION. STUDY BY FLOW CYTOMETRY

N. Fernandez Mosteirin, C. Salvador Osuna, A. Aranda, M. Torres, J.G. Galache, J.J. Gimeno, A. Godoy, N. Padron, F. Sevil, B. Soria, J.F. Lucia, M. Giralt

Hospital Universitario Miguel Servet, Zaragoza, Spain

Aims. To design a protocol to stablish normal parameters of platelet (Plt) activation in our population using whole blood flow cytometry assays, and study the variability of responsiveness to an agonist among healthy people. *Material and Methods.* 25 healthy blood donors were included in the study, none of them showed diabetes, hypertension or pharmacological treatment. Blood samples were drawn using standard phlebotomy techniques to obtain a 4.5 mL vial containing 3.8% citrate with a 16G needle. Samples were collected in conditions to avoid stasis and prevent artifactual plt activation. To avoid possible observer bias, blood samples were coded and blinded. The blood was carefully mixed and diluted in Tyrode´s buffer (not diluted in monocytes (mon) and neutrophils (nt)-binding platelets study), and then separated into six polypropylene tubes (3 baseline and 3 adding ADP). To detect plt response to an agonist (activation) we added a 100 _M ADP concentration. The surface expression of plt receptors was determined by flow cytometry using the following monoclonal antibodies: anti-CD41 (Gp IIb/IIIa) FITC and PE, anti-CD62p (P-selectin) PE, anti-CD11b FITC, anti-CD14 PC5 (Immunotech, Marseille, France) and PAC-1 (activated Gp IIb/IIIa) FITC (BD Biosciences, San Jose, CA, USA). The samples were analized on a Coulter™ EPICS XL-MCLTM. All parameters were collected in list mode fields and then analyzed. PAC-1 was expressed in mean fluorescence units (MFI) of total plt population. CD62p and leukocytes positive for plts were expressed in percentage (%). Descriptive statistics and correlation test were also studied. *Results.* Blood samples from 3 females and 22 males with a median age of 40 years (range: 23-61) were studied. See descriptive statistics in table below. We found a significative correlation coefficient (r=0.837) between P-selectin expression and PAC-1 binding. We didn´t find correlation between age and activation parameters. *Conclusions.* 1) Our data indicate that ADP induced plt activation varies considerably from one individual to one, as observed by other groups. 2) In healthy adults we demonstrate that the expression of P-selectin (α granules release) is strongly correlated to the binding of PAC-1 (conformational change) according to the results of other series.

Table 1.

	Median	*Range*	*Std. Dev.*		*Median*	*Range*	*Std. Dev.*
PAC-1 (MFI)	0.4	0.3-0.7	0.1	Plt-Mon (%)	25	9.7-51.9	11.4
PAC-1 ADP (MFI)	2.3	0.7-5.7	1.3	Plt-Mon ADP (%)	50.1	16.4-96.7	18.7
ΔPAC (MFI)	1.8	0.3-5.2	1.2	ΔPlt-Mon ADP (%)	23.7	0.8-68.9	16
CD62p (%)	11.2	4.4-18.0	3.2	Plt-Nt (%)	8.5	3.5-38.6	7.6
CD62p ADP (%)	26	11.3-57.4	11.2	Plt-Nt ADP (%)	17.3	8.5-58.5	14.5
ΔCD62 p (%)	14	1.7-44.1	10	Δ Plt-Nt (%)	8.7	0.8-44.5	10.1

0931

EFFICACY AND SAFETY OF IGIV3I GRIFOLS (HUMAN INTRAVENOUS IMMUNOGLOBULIN) IN PATIENTS DIAGNOSED WITH IMMUNE THROMBOCYTOPENIC PURPURA

A. Vidaller,[1] I. Alberca,[2] A. Julia,[3] V. Sandoval,[4] J. Sierra[5]

[1]*Hospital Universitario de Bellvitge, Barcelona, Spain;* [2]*Hospital Universitario de Salamanca, Salamanca, Spain;* [3]*Hospital General Vall dHebron, Barcelona, Spain;* [4]*Hospital de Leon, Leon, Spain;* [5]*Hospital de la Santa Creu i Sant Pau, Barcelona, Spain*

Intravenous immunoglobulin (IVIG) therapy is an useful treatment in patients with chronic idiopathic/immune thrombocytopenic purpura (ITP) in whom the platelet count has to be rapidly increased to prevent bleeding or prior to surgery. IGIV3I Grifols is a highly purified, unmodified human IgG product whose manufacturing process follows the same basic principles of Flebogγ™ (another IVIG manufactured by Grifols in clinical use since 1992). The main differences between both processes are how purification steps are sequentially arranged, and the introduction of two specific steps to inactivate/remove any potential contaminating pathogen (solvent-detergent treatment and sequential nanofiltration through 35 and 20 nm pore size filters), as additional viral elimination steps to pasteurization, already present in Flebogγ™. Essentially IGIV3I Grifols is prepared from fraction II+III of Cohn's fractionation and the purification of IgG is performed by means of sequential polyethyleneglycol precipitations. Further reduction down for all remaining potential impurities is achieved through ion exchange chromatography with DEAE resins. Finally, it is formulated with sorbitol (5%) as stabiliser. An open, prospective, multicentre study was planned to investigate the efficacy and safety of IGIV3I Grifols in 20 adult patients with chronic ITP (at least 6 months after diagnosis). It was designed in accordance with the European Union guidelines from the EMEA for such trials. A total of 19 adult patients with chronic ITP in acute phase (platelet counts below 20×10^9/L) were treated with the study drug. Patients received a total dose of 0.4 g/kg body weight for 5 consecutive days. Primary efficacy endpoint was the proportion of patients who reach a platelet count equal or > 50×10^9/L. The time taken for the platelet count to reach the target level since first dose of IGIV3I Grifols and duration of response

were also determined within 1 month after first infusion. Regression of haemorrhages was documented during the first 14 days of follow-up. Safety parameters including adverse events (AEs), laboratory determinations and vital signs were regularly monitored. The follow-up of patients ended 3 months after first dose of IGIV3I Grifols to determine any change in viral markers for HIV, HCV, HBV and HAV. An interim analysis from available results of 8 out of 20 patients is presented. A patient was withdrawn from the study after confirmation of secondary thrombocytopenia. A total of 5 patients (71%) responded to the study drug. The mean time to platelet response was equal or <3.4 days (SD=1.1) and the mean duration of response was equal or >10.0 days (SD=7.9). Haemorrhagic symptoms compared with baseline improved in all seven patients (100%). Five out of 8 treated patients presented a total of 12 AEs potentially related to the study drug (8 mild and 4 moderate). Headache and fever (4 cases each), changes in blood pressure (3 cases) and decrease in heart rate (1 case) were AEs potentially related to study drug. The results show that IGIV3I Grifols is effective, well tolerated and safe in the treatment of adult patients with chronic ITP.

0932

RITUXIMAB IN REFRACTORY IDIOPATHIC THROMBOCYTOPENIC PURPURA

C.S. Santoro, G. De Angelis, A. De Vellis, A. Guarini, M.L. Milani, A. Rago, R. Foà, M.G. Mazzucconi

Hematology, Rome, Italy

Background. Rituximab, a chimeric anti-CD20 monoclonal antibody effective in B-cell depletion, may be useful in autoimmune disorders by interfering with the production of auto-antibodies. *Aims*. To investigate the efficacy of Rituximab in patients with resistant ITP. *Patients and methods*. Eleven adult ITP patients (2 males, 9 females; median age 46.3 years, 28.6-67.8) were treated with Rituximab (375 mg/m^2/weekly for four doses). Median time between diagnosis and start of Rituximab was 4.1 years (0.2-33.1 months). All patients had already received at least two lines of therapy (median 3; 2-6): prednisone, pulsed high-dose dexamethasone, azathioprine, immunoglobulins, interferon or splenectomy. At the start of Rituximab, the median platelet count was 10×10^9/L (3-20×10^9/L). Response definitions: complete response (CR), platelet count ≥150×10^9/L; partial response (PR), >50 <150×10^9/L; minimal response (MR), >20 ≤50×10^9/L; no response (NR) ≤20×10^9/L. After completing therapy, patients were evaluated for platelet count after 1 and 3 months, and thereafter every 3 months until relapse or start of a different treatment. Peripheral blood B lymphocytes were evaluated by flow-cytometry as CD19$^+$ cells before treatment, 1 and 3 months after stopping therapy, and then every 3 months up to recovery. *Results*. One month after Rituximab therapy, 5 responses (1 CR, 3 PR, 1 MR; 45%) and 6 NR (55%) were observed. Two relapses occurred 5 and 18 months after response. The median follow-up of all treated patients is 8.7 months (1.8-31.1), while the median follow-up of all responsive patients is 13.7 months (2.6-18.7). Before starting therapy, 9/11 cases were evaluable for flow-cytometry studies. The median baseline value of peripheral blood CD19$^+$ B-cells was 128×10^6/L (58-371). One month after completing therapy, 6/8 evaluable cases showed absence of CD19+ cells and 2/8 showed a count of 9 and 4.4×10^6/L CD19$^+$ cells, respectively. At the last available control (median follow-up of 11 months; 1-28), 8/9 patients had still not recovered the baseline CD19$^+$ cell count (median value: 6×10^6/L; 0-295). The following side effects were observed: 3 cases of papulosquamous dermatitis, 1 case of fever and 1 case of fever and dermatitis. *Conclusions*. Five/11 (45%) ITP patients had an early response to Rituximab (1 CR, 3 PR, 1 MR), that persisted in 3 cases. No late responses were observed. The response was independent from the post-therapy CD19$^+$ cell. No serious infections were observed during the clinical follow-up. No patient had to stop therapy because of severe side effects.

0933

ISOLATED THROMBOCYTOPENIAS: 'NATURAL' HISTORY OF 281 CASES

G. Frigerio, C. Casartelli, M. Duro, G. Scognamiglio, F. Alberio

Ospedale Valduce, Como, Italy

Aims. evaluate the outcome of isolated thrombocytopenias (patients without any hematological disorder other than thrombocytopenia and without any known underlying disease possible cause of thrombocytopenia), the probability of hemorrhagic events and need of therapy. Methods. A series of 281 patients with isolated thrombocytopenias - 105 men (37%) and 176 women (63%) were followed up as outpatients for range of 1-310 months (median 23 - mean 44). The platelet count (PLT) at diagnosis ranged from 1000 to 137000/mm^3 (median 88000 - mean 78000). The clinical classification at diagnosis was: 25% (95% C.I.: 20.0 - 30.4) severe - PLT under 50000/mmc - 36% (C.I.: 30.3 - 41.9) moderate - 51000-100000/mm^3 - 39% (C.I.: 33.1 - 44.8) mild - 101000-150000/mm^3; at last follow up these percentages changed to: 10% severe - 29% moderate - 33% mild and 28% normal (> 150000/mm^3). Pseudothrombocytopenias resulted in 31 patients (11% - C.I. 7.6-15.3; 9 men (8.6%) and 22 women (12.5%); p=0.412 - N.S.) A positive anamnesis for drugs potentially lowering PLT resulted in 40 patients (14.2%; C.I.: 10.4 - 18.9): 29 women (16.5%) - 11 men (10.5%) - p=0.224 - N.S. The drugs involved were: ASA or FANS in 16 cases (40%) Estrogen-gestagen combinations in 6 (20%) and various in 16 (40%). Hemorrhagic symptoms, all non fatal, developed in 28 (10%) patients (7 men: 6.7% - 21 women - 11.9%; p=0.22 - N.S.): 17 cutaneous - 9 mucosal - 1 prolonged hypermenorrea - 1 severe digestive bleeding. The PLT at time of hemorrhagic symptoms ranged from 1000 to 124000/mmc (mode 1000/mm^3 - median 12400/mm^3). In 20 patients (71%) PLT was < 30000/mmc and therapy was performed only in these patients. Only 3 patients (1% - 2 women and 1 man) presented at diagnosis with hemorrhagic symptoms and positive anamnesis for drugs. Therapy was performed in 49 cases (17%): one women splenectomized at diagnosis - 1 man treated with interferon and 47 cases with steroids: in 29 patients (10% of all cases) up to 6 months and in 18 (6%) longer than 6 months (for 6 patients - 2% - over 24 months). Three women refractory to steroid therapy were splenectomized; one woman was refractory to steroids, vinca alkaloids, high doses immunoglobulins i.v. and splenectomy. After therapy 21 patients (43%) reached normal PLT >150000/mmc; in 9 (18%) persisted mild thrombocytopenia, in 10 (21%) moderate and 9 (18%) severe. In 5 patients (1.8%; C.I.: 0.06'4.1) was successively diagnosed autoimmune disease (all with clinical presentation of only thrombocytopenia). *Conclusions.* The overall probability of hemorrhagic events in these patients is 10% (95% C.I.: 7-15) and the probability of severe hemorrhagic event of 0.4% (95% C.I.:0.01-2); none fatal event has occurred (95% C.I.:0-1.1). The outcome of isolated thrombocytopenias appear very favourable and therapy can be performed in a minority of cases (17%)

0934

IDIOPATHIC THROMBOCYTOPENIC PURPURA IN CHILDHOOD: REVIEW OF 160 CASES

G. Martinova, O. Muratovska, S. Glamocanin, Z. Antevska-Trajkova, B. Coneska-Jovanova, S. Koceva

University Children's Hospital, Skopje, Macedonia

Idiopathic thrombocytopenic purpura (ITP) is one of the most common acquired bleeding disorders in childhood. Usually it is a benign self-limited disease that occurs in previously healthy children. The purpose of the present study was to review the clinical course and management of all children with ITP admitted over eight-year period to the Pediatric Clinic in Skopje. One hundred and sixty cases were identified indicating an incidence of 4/100000 children under 15 years. The sex ratio (female/male) was 1,2:1. 95 patients (59%) were at the age between 2-10 years, 36 (22,5%) under 2 years and 30 (18,5%) older than 10 years. 88 (55%) presented with cutaneous signs only, and 65 (40,6%) had plus mild mucous membrane bleeding. Just 12 (7,5%) had serious bleeding symptoms: 8 (4,8%) nosebleeds requiring nasal packing, 3 (1,8%) increased menstrual bleeding and 1 (6,2%) macrohematuria. No patient suffered an ICH or severe bleeding requiring transfusion. The mean platelet count on admission was 21,4×10^{9}L, lowest count 3×10^{9}L. Bone marrow aspiration was performed almost in all cases. Initial management consisted of no drug treatment in 9 patients (5,6%), intravenous immunoglobulins (IVIG) in 17 (10,6%) and glucocorticosteroids (GS) in 134 (83,8%). IVIG were used just in infants as they were expensive form of treatment. All patients improved regardless of the management strategy used. The mean length of stay in hospital in the period between 1998-2001 was 14,5 days, and between 2002-2005 7,9 days. We consider it could be reduced more and majority of patients, particularly those with cutaneous bleedings only, could be treated as outpatients. *Conclusions.* Children presenting with ITP have a low incidence of bleeding complications and a good response to standard treatment with oral prednisone. Many of these patients can be managed as outpatients.

0935

AUTOIMMUNE THROMBOCYTOPENIA: CLINICAL AND HEMATOLOGICAL OUTCOME OF PATIENTS AFTER DANAZOL ADMINISTRATION AS FIRST LINE OR REFRACTORY DISEASE TREATMENT

E. Kyriakou,[1] CS Chrysohoou,[2] D.M. Dimou,[2] G.P. Grecka,[2] Z.C. Zouvelou,[2] G.G. Georgiou,[2] N.D Ntoufexis,[2] K.M.C Kyrtsonis,[2] P.P. Panayiotidis[2]

[1]*'Laikon' University Hospital of Athens, Athens, Greece;* [2]*Laikon University Hospital of Athens, Athens, Greece*

Introduction. First line treatment of Autoimmune thrombocytopenia (AT) consists of corticosteroids and intravenous immunoglobulin. In refractory cases, splenectomy is indicated. In older patients or in those with a worse performance status, long term corticosteroid administration and splenectomy may be harmful and alternative treatments are under evaluation. Danazol is an androgen with mild, rare and reversible adverse events, which plays a role in AT therapy. *Aim of the study*. The aim of our study was to estimate the effectiveness and safety of danazol treatment in newly diagnosed or relapsed patients with AT. *Patients and Methods.* 35 patients (23 male/12 female) suffering from AT (30:ITP, 4:MDS/ITP, 1:low grade-NHL/ITP) were studied from 2002 to date. Median age was 65 years (23-92 years). 21 patients were >60yo and newly diagnosed, while 14 patients were <60 years and relapsed. Patients over 60 years with newly-diagnosed AT, no severe bleeding and Plts>20000/μL were treated with Danazol alone (200 mg q8h p.os. daily). The same-aged patients with Plts<20000/μL or/and with severe bleeding were initially treated with an induction regimen (IR) (dexamethasone 40 mg/day × 4days iv or/and IVIG 0.4g/kgBW/day × 5days iv) aiming at rapid platelet count restoration, because of slow response to danazol *per se*. Patients below 60 years with relapsed AT after standard treatment, were treated with Danazol alone (200 mg q8h p.os. daily). Patients who relapsed under danazol treatment, were treated with IR again or low dose methylprednisolone (4-8 mg per os daily) was added to danazol. After second rapid platelet count restoration, patients continued danazol monotherapy. First and second response to danazol was separately estimated in those patients. Average follow up period was 19 months, median was 7 months. Response criteria were defined as follows: Clinical remission: absence of bleeding manifestation : complete hematological remission (CR): Plts≥140000/μL; Partial hematological remission (PR): Plts 50000-139000/μL; minimal hematological remission (MR): Plts 20000-50000/μL; no hematological remission (NR): Plts<20000/μL. *Results.* The overall response rate in patients treated with danazol alone (23/35) was 56,5% (CR 39,1%, PR 17,4%).

Table 1.

	Initial treatment	*Treatment options*	*CR*	*PR*	*MR*	*NR*	*NA*	*SUM*
	Initial IR**, continue with danazol alone	Danazol alone	4	3	–	–	–	7
		Interim response to danazol→ relapse→ IR→continue with danazol alone	2	2	–	–	1	5
>60 years, first diagnosed	Danazol alone	Danazol alone	2	2	–	1	–	5
		Interim response to danazol→ relapse→ IR→continue with danazol alone	1	3	–	–	–	4
<60 years, relapsed*	Danazol alone	Danazol alone	7	2	–	–	–	9
		Interim response to danazol→ relapse→ IR→continue with danazol alone	3	1	–	1	–	5
		SUM	**19**	**13**	**–**	**2**	**1**	**35**

**:after standard initial treatment; **: IR: induction regimen (see text).*

In patients over 60yo treated with primary IR and continued with danazol alone (12/35) the response rate was 58,3% (CR 33,3%, PR 25%). In patients with interim response to danazol -either alone or after initial IR administration- 40% (14/35) relapsed during follow up. These patients were treated with a second IR and returned to danazol monotherapy. 86% of them achieved second response to danazol (CR 43%, PR 43%) (Table 1). Co-administration of low dose methylprednisolone induced the response rate to danazol without causing standard corticosteroid adverse events. Average duration of response to danazol was 19 months, median was 7 months. The median time to response to danazol was 1 month. 97% (34/35) of patients showed clinical response and 31,4% (11/35) had adverse events due to danazol treatment: 9/35 had elevated liver enzymes (2/9 drug cessation due to severe reversible transaminasemia), 2/35 had mild renal function impairment. *Discussion*. Danazol is a safe and cheap treatment for autoimmune thrombocytopenia, as second line therapy in young relapsing patients, or as first line treatment in older patients, because long-term corticosteroid treatment is avoided with this schedule.

0936

DESCRIPTIVE EPIDEMIOLOGY OF IMMUNE THROMBOCYTOPENIC PURPURA IN THREE EUROPEAN COUNTRIES

J. Satia,[1] J. Acquavella,[2] J. Hollowell,[2] M. Rutstein[2]

[1]*University of North Carolina, Chapel Hill, USA;* [2]*Amgen Inc., Thousand Oaks, USA*

Backgrounds. The incidence and prevalence of immune thrombocytopenic purpura (ITP) has not been well characterized to date. *Aims.* To characterize ITP incidence and prevalence in three European countries overall and according to sex. Also, to determine whether ITP incidence and prevalence rates are increasing. *Methods.* Incident and prevalent cases were identified from databases from three countries: United Kingdom (General Practitioners Research Database (GPRD) 1990 through 2000), Germany (IMS Disease Analyzer Mediplus 1994 through 2003), and Netherlands (PHARMO database 1991-2003). GPRD and IMS include general practice physicians chosen such that their patients are representative of their respective countries. IMS also includes specialists. PHARMO contains hospitalization data from the National Medical Registry covering all hospital admissions in the Netherlands. Dutch population counts were obtained from Statistics Netherlands. ITP diagnoses were identified using the relevant coding system/codes for each database: ICD-9 287.3 (PHARMO), ICD-10 D693 (IMS Disease Analyser Germany), and Read and OXMIS codes corresponding to an ITP diagnosis (GPRD). Incidence rates include only first time diagnoses, whereas prevalence rates include new and recurrent diagnoses. *Results.* The average annual incidence rate in the UK was 3.0 per 100,000 person years (95% confidence interval (CI) 2.7 to 3.3). Rates were fairly stable over time, ranging from 1.0 per 100,000 person years in 1998 to 3.7 per 100,000 in 1991. Average incidence, unadjusted for age, was 3.4 per 100,000 person years for women and 2.5 per 100,000 for men. Prevalence rates ranged from a low of 2.1 per 100,000 person years in 1990 and 2000 to a high of 8.1 per 100,000 in 1997. For Germany, average annual incidence was 2.7 per 100,000 person years (95% CI 1.7 to 4.1), ranging from 1.6 per 100,000 in 1996 to 5.1 per 100,000 in 1994. Incidence rates were comparable for German men and women. Prevalence ranged from 2.8 per 100,000 person years in 1999 to 7.3 per 100,000 in 1994. In the Netherlands, average annual incidence was 1.9 per 100,000 person years (95% CI 1.8, 2.0) varying little from 1991 through 2003 (1.7 to 2.1 per 100,000 person years). Average incidence was slightly higher for women than for men (2.2 per 100,000 and 1.8 per 100,000 person years, respectively). Annual prevalence ranged from 1.9 per 100,000 to 2.4 per 100,000 person years. *Summary/Conclusions.* ITP incidence and prevalence rates were less than 5 and 10 per 100,000 person years, respectively, in three major European countries. Incidence rates were higher for women than men in the UK and the Netherlands, but not in Germany. Rates did not increase during the period 1990 through 2003. These analyses of general practice and national medical databases provide a robust picture of recent ITP incidence and prevalence with a degree of precision lacking in previous evaluations of this relatively rare condition.

0937

THE SALVAGE TREATMENT WITH CYCLOSPORIN A IN IDIOPATHIC THROMBOCYTOPENIC PURPURA

G.D. Gaman, A.M. Gaman

University of Medicine and Pharmacy, Craiova, Romania

Backgrounds. Physicians face therapeutic dilemas when patients became resistant to known treatment in life-threatening conditions. A review of the literature shows a lack of comprehensive information on the clinical use of Cyclosporin A in the treatment of idiopathic thrombocytopenic purpura (ITP). *Aims.* To verify the usefulness of Cyclosporin A therapy in refractory ITP. *Method*. Study was carried out on long-term treatment (median 36 months) with Cyclosporin A (3 mg/Kg/day) in 14 selected adult patients with chronic, severe ITP resistant to all the usual therapies. *Results.* A median follow-up of 26,2 months shows that Cyclosporin A treatment obtained an improvement in 10 out of 14 patients (71%): 9 patients (64%) achieved a complete response and one (7%) a partial response. Four patients (28%) were unresponsive. In 5 patients (50%) the discontinuation of Cyclosporin A was successful, maintaining the remission over time, without further therapy. In the remaining responsive patients, the remission was dependent on continued of drug. The Cyclosporin A intolerance was slight in spite of long-term treatment and no nefrotoxicity occured. Conclusions. Our study shows the safety and efficacy of Cyclosporin A therapy in resistant ITP. Because the potential role in second neoplasia appearance and the well known teratogenic role of this immunosuppressor, cyclosporin A will be done only in resistant ITP cases (dramatic clinical cases).

Myelodysplastic syndromes II

0938

EFFECT OF CA2 ANTI-TUMOR NECROSIS FACTOR (TNF) α ANTIBODY THERAPY ON HAEMOPOIESIS OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES

A. Boula,[1] M. Voulgarelis,[2] S. Giannouli,[2] G. Katrinakis[1], M. Psyllaki,[1] C. Pontikoglou,[1] F. Markidou,[1] G. Eliopoulos[1], H.A. Papadaki[1]

[1]*University of Crete School of Medicine, Heraklion, Greece;* [2]*Pathophysiology Dept of Medical School, Athens, Greece*

Backgrounds. TNFα plays a prominent role in the pathophysiology of myelodysplastic syndromes (MDS) by inducing apoptotic death of bone marrow (BM) haemopoietic cells directly and/or indirectly by upregulating Fas antigen expression. *Aims.* To explore the biological and immunoregulatory effect of the treatment with the anti-TNFα monoclonal antibody cA2 on BM progenitor/precursor and stromal cells and lymphocyte subsets as well as the clinical response in MDS patients. *Methods.* Ten low-intermediate risk MDS patients received intravenously cA2 (3 mg/kg) at weeks 0, 2, 6 and 12. At baseline and end of the treatment, we evaluated: (a) The BM stem/progenitor cell reserve and function using a limiting dilution assay for the enumeration of the long-term culture initiating cells (LTC-ICs) in the $CD34^+$ cell fraction, clonogenic assays for the quantification of the colony-forming cells (CFCs) in the BM mononuclear (BMMCs) and $CD34^+$ cell fraction, and flow-cytometry for the evaluation of the percentages of $CD34^+$ cell subpopulations and the proportion of apoptotic (7-aminoactinomycin-D positive; 7-AAD^+) and Fas^+ cells in the $CD34^+$ cell fraction. (b) The activation status of BM and peripheral blood (PB) lymphocytes using flow-cytometry. (c) The BM stromal cell function to sustain the autologous and normal haemopoiesis using standard long-term BM cultures (LTBMCs) or irradiated LTBMCs recharged with normal $CD34^+$ cells. Clinical responses were evaluated according to standardized criteria. *Results.* The number of LTC-ICs cells did not change significantly following treatment compared to baseline. Of the CD34+ cell subpopulations, a significant increase was obtained in the proportion of $CD34^+/CD33^+$ myeloid progenitor cells compared to baseline (p=0.0192). The proportions of $CD34^+/CD61^+$ megakaryocytic and $CD34^+/CD71^+$ erythroid progenitor cells and the percentage of $GlycoA^+$ erythroid precursor cells did not change significantly. The number of CFCs obtained by BMMCs and $CD34^+$ cells increased significantly following treatment compared to baseline (p=0.0399 and p=0.0304, respectively). This increase was due to the improvement of CFU-GM (granulocyte-macrophage colony forming units) and CFU-Meg (megakaryocytic colony forming units) numbers in the BMMCs (p=0.0298 and p=0.016, respectively) and $CD34^+$ cells (p=0.0441 and p=0.002, respectively) post-treatment. The proportion of apoptotic (7AAD+) cells and the percentage of Fas^+ cells in the $CD34^+$ cell fraction decreased significantly post-therapy compared to baseline (p=0.0215 and p=0.0344, respectively). The proportions of activated BM and PB T-cells decreased significantly after treatment as was indicated by the percentage of Fas+ $HLA\text{-}DR^+$, $CD25^+$, $CD38^+$ and $CD69^+$ cells in the $CD3^+$ cell fraction. Treatment with cA2 reduced significantly TNFa levels in LTBMC supernatants (p=0.0043) and improved significantly the haemopoiesis supporting capacity of LTBMC adherent cells. Two patients displayed minor haematologic responses while the remaining displayed stable disease with no disease progression. *Summary-Conclusions.* Treatment with cA2 down-regulates the Fas-mediated apoptotic depletion of BM $CD34^+$ cells, increases the clonogenic potential of haemopoietic progenitor cells, ameliorates the hemopoiesis supporting capacity of BM stroma and decreases the proportion of activated T-lymphocytes in both BM and PB. The encouraging biological insights from cA2 administration may appear useful in conducting further clinical trials using cA2 for selected MDS patients particularly those with evidence of immune-mediated inhibition of haemopoiesis.

0939

RESULTS OF CLONALITY ASSAY AND MEASUREMENT OF APOPTOTIC RATE AND TELOMERE LENGTH SUPPORT USEFULNESS OF SEPARATION OF REFRACTORY CYTOPENIA FROM REFRACTORY ANEMIA AS A DISTINCT SUBTYPE OF EARLY MDS

J. Cermak,[1] M. Belickova,[1] L. Marinov,[2] Z. Sieglova,[2] K. Michalova[2]

[1]*Institute of Hematology, Praha, Czech Republic;* [2]*Institute of Haematology, Praha, Czech Republic*

Background and aim of the study. The degree of clonality, telomere length

and the rate of apoptosis represent laboratory markers that may be related to the progression of pathological clone in patients with myelodysplastic syndrome (MDS). In this study we investigated these markers in patients with different subtypes of MDS. Methods. X-chromosome inactivation pattern clonality assay based on PCR amplification of polymorphic short tandem repeats of the human androgen receptor (HUMARA) gene was performed in granulocyte, CD14+ and CD3+ cell subpopulations isolated from bone marrow and peripheral blood of 58 females with primary MDS and 20 healthy controls. The results were compared with measurement ot the telomere length by Terminal Repeat Fragment (TRF) method and with apoptotic rate of CD34+ and GlyA+ subpopulations assessed by flow cytometry (Annexin V. and TUNEL methods). Results. In 19 patients with advanced MDS (RAEB,RAEB-T,CMML according to the FAB classification) clonal granulocyte and CD14+ cell subpopulations (allele ratio ≥ 9:1) were present in bone marrow and peripheral blood of 74% and 87% of patients, respectively. Shortened telomere length (TRF < 7,5 kbp) and low rate of apoptosis of CD34+ bone marrow cell subpopulation were present in all patients with advanced MDS. In patients with early MDS, clonal patterns of hematopoiesis were present only in 2 out of 17 patients (12%) with RA, RARS or 5q- syndrome according to the WHO classification. On the other hand, clonal granulocyte or CD14+ cell subpopulations were present in bone marrow or peripheral blood of 20 out of 22 patients (90%) with RCMD (according to WHO criteria). In accordance with these results, 80% of patients with early MDS and clonal granulocyte cell subpopulations exhibited low apoptotic rate of CD34+ bone marrow cells (5-12%). On the contrary, 80% of patients with non-clonal cells had increased apoptotic rate of CD34+ cells (30-80%). Reduced telomere length was found in 71% patients with clonal cell subpopulations v.s. 45% patients with non-clonal cells. Median survival of patients with early MDS and clonal cells was 62,5 months v.s. 47,8 months in those with non-clonal cells (p=0.05) and 65,7 months in RA patients v.s. 50,0 months in RCMD patients (p=0.05). Conclusions. The results confirm our preliminary observations suggesting that RCMD represents a separate clinical and laboratory entity with adverse prognosis which is distinct from RA and support hypothesis of multistep pathogenesis of MDS, where dysplasia limited to erythropoiesis may represent an early step and multilineage dysplasia is a subsequent step reflecting progression of pathological clone.

Funding: The study was supported by scientific program MZO 00023736 from Czech Ministry of Health.

0940

INHIBITION OF THE MKK3-P38 MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY IS REQUIRED FOR NEUTROPHIL DIFFERENTIATION OF HUMAN CORD BLOOD DERIVED CD34+ CELLS

C. Geest,[1] M. Buitenhuis,[1] E. Vellenga,[2] P.J. Coffer[1]

[1]*UMC Utrecht, Utrecht, Netherlands;* [2]*UMC Groningen, Groningen, Netherlands*

Patients with myelodysplasic syndromes (MDS) suffer from recurrent bacterial infections as a result of differentiation defects of the neutrophil lineage. While limited number of genetic defects of MDS progenitor cells has been described, the defective intracellular signal transduction pathways modulating these developmental defects remain undefined. Mitogen-activated protein (MAP) kinase cascades play a key role in regulating a plethora of cellular processes. They typically are organized in a three-kinase architecture consisting of a MAPK, MAPK activator (MKK or MAPK kinase), and a MKK activator (MAPK kinase kinase). The p38 MAPK pathway mediates a wide variety of cellular processes in response to extracellular stimuli such as UV light, osmotic shock, inflammatory cytokines and growth factors and it has been shown that MKK3 and MKK6 are the main MKKs activating p38. Although p38 has been demonstrated to regulate differentiation in several cell types, its role in regulating neutrophil development in both normal as well as in defective MDS granulopoiesis remains to be investigated. *Aims*. The aim of this study was to investigate the role of the p38 MAPK signalling module in neutrophil differentiation and to determine whether p38 MAPK signalling may play a role in aberrant neutrophil development in MDS Mononuclear cells were isolated from umbilical cord blood using a ficoll-paque solution and MACS immunomagnetic cell separation was used to isolate CD34+ cells. Cells were cultured in IMDM supplemented with 9% serum and differentiation towards neutrophils was induced upon addition of SCF, FLT-3, GM-CSF, IL-3 and G-CSF. After 6 days of culture, only G-CSF was added. The specific p38 pharmacological inhibitor SB203580 was freshly added every 3 - 4 days during culture. Retroviral transduction experiments were performed at day 2 and 3 to ectopically express constitutively-active MKK3. During neutropoiesis cells were counted every 3 days and the percentage of apoptotic cells was determined by analyzing Annexin-V positive cells. The morphology of differentiating neutrophils was analyzed by May-Grunwald Giemsa staining. Neutrophil differentiation was also analyzed by intracellular staining of lactoferrin. *Results*. Inhibition of p38 during neutrophil differentiation by SB203580 resulted in an approximately 30% increase in proliferation, which was not due to enhanced survival. In addition, after 17 days of neutrophil differentiation, approximately 45% of SB203580 treated cells consisted of banded or segmented nuclei, whereas only 25% of the control cells were characterized as mature neutrophils. Conversely, ectopic expression of constitutively-active MKK3 resulted in a 40% reduction in proliferation compared to eGFP alone, which was not due to an increase in apoptosis. Transduction of cells with eGFP resulted in approximately 35% mature neutrophils after 17 days of culture, whereas ectopic expression of constitutively-active MKK3 resulted in an almost complete block in neutrophil differentiation. *Conclusions*. These results demonstrate that regulating p38 activity is critical for neutrophil development. Inhibition of p38 activity is necessary for terminal differentiation of CD34+ progenitor cells. Since neutrophil development is blocked in patients with MDS and downregulation of p38 activation is required for normal neutrophil differentiation, it can be hypothesized that deregulation of p38 activation might be involved in aberrant neutrophil maturation in MDS.

0941

MITOCHONDRIAL INVOLVEMENT IN 5-AZACYTIDINE-INDUCED APOPTOSIS

R. Khan, J. Schmidt-Mende, V. Gogvadze, M. Grövdal, A. Forsblom, M. Hassan, B. Zhivotovsky, E. Hellström-Lindberg

Karolinska Institute, Stockholm, Sweden

Backgrounds. Although 5-azacytidine is the only drug approved by the FDA for high-risk MDS, its mode of action has not been well characterized. Aim: The aim of the study was to investigate the mechanisms of 5-azacytidine-induced cytotoxicity in myeloid P39 cells in order to optimize the use of this drug. *Methods.* Cells were incubated with 0.1-2 μM of 5-azacytidine for 4-48 hours. Nuclear apoptotic morphology was assessed by light microscopy. Processing of caspases, cytochrome-c release into the cytosol, cleavage of PARP, expression and cleavage of Bcl-2 family proteins were analyzed by Western blot. Loss of mitochondrial membrane potential was estimated by FACS analysis using the fluorescent dye TMRE. *Results.* 5-azacytidine induced apoptosis in P39 myeloid cells. Dose-dependent activation of PARP as well as processing and activation of caspase-2 were observed. Furthermore cleavage of Bcl-2-family proteins, namely, Bcl-2, Bax and Bid was documented. Mitochondrial involvement, characterized by loss of mitochondrial membrane potential and release of cytochrome-c into the cytosol was detected. Although caspase-3 activation occurred, various inhibitors of caspase-3 (DEVD-fmk), -2 (VDVAD-fmk), -8 (IETD-fmk), -9 (LEHD-fmk) and pan-caspase inhibitor (zVAD-fmk) did not block apoptosis. Moreover, inhibitors of the poly (ADP-ribose)polymerase, PARP, (nicotinamide and 3-aminobenzamide) only partially block apoptosis (25-41% and 31-43% decrease, respectively). *Conclusion*. 5-azacytidine activates PARP, which in turn induces mitochondrial dysfunction. At the mitochondrial level this compound suppresses anti-apoptotic properties (cleavage of Bcl-2) and increases pro-apoptotic activities (cleavage of Bax and Bid). These events result in the loss of mitochondrial membrane potential and release of cytochrome-c into the cytosol. As PARP inhibitors only partially block loss of mitochondrial membrane potential, and caspase inhibitors did not have any effect on any of apoptosis manifestations, we conclude that 5-azacytidine induces cell death via activation of caspase-independent pathway. It seems that caspase activation plays a secondary role in this process.

0942

CLINICAL CHARACTERISTICS AND TREATMENT OF 217 NEW MDS PATIENTS DURING THE YEAR 2005 IN A TERTIARY REFERRAL CENTER

U. Germing,[1] S. Knipp,[1] C. Strupp,[1] A. Kuendgen,[1] S. Balleisen[1], H.K. Habersang,[1] F.F. Fox,[1] A.M. Aivado,[1] C.A. Andresen[1], G.K. Kobbe,[1] B. Hildebrandt,[2] N. Gattermann,[1] R. Haas[1]

[1]*University of Düsseldorf, Düsseldorf, Germany;* [2]*Inst. of Human Genetics, University of Düsseldorf, Düsseldorf, Germany*

Backgrounds. Heterogeneity of myelodysplastic syndromes not only relates to the biology of the disease but also to the spectrum of patients

seen by different health care providers. Aims. We gathered data on patient characteristics and treatment of 217 new MDS patients seen in our medical center during the year 2005. *Methods.* All MDS patients treated either in the hematology outpatient clinic or on the wards were documented. A diagnosis of MDS was made according to the standards of the German MDS Registry in Düsseldorf, including central morphology. Patients were followed for any kind of complication, disease progression, and therapeutic intervention during the year 2005. *Results.* In 2005, a total of 217 patients were seen at our institution. In 90 patients (41%) the diagnosis of MDS was made during that year, either before or after referral. 93% had primary MDS, 7% were diagnosed as treatment-related MDS. The distribution among MDS types was: 10 RA, 4 RARS, 64 RCMD, 26 RCMD-RS, 26 RAEB I, 33 RAEB II, 25 CMML, 6 patients with 5q- syndrome, and 23 patients with RAEB-T. A karyotype analysis was available in 82% of patients (n=179). 100 patients (56%) pre-sented with a normal karyotype. According to the International Prognostic Scoring System (IPSS), 25% of the patients belonged to the low-risk, 36% to the Intermediate-1, 24% to the Intermediate 2, and 15% to the high-risk group. A 5q- anomaly. Either as sole abnormality or as part of a more extensive derangement, was found in 17 patients. There were 121 males and 96 females. 128 (59%) patients were treated in our outpatient clinic only, with a median number of 4 consultations with the doctor (1-67). 89 (41%) pa-tients were admitted to the hospital, 68 of whom (31%) were treated on the ward as well as in the outpatient clinic. Reasons for hospitalization were disease complications like infections, hemorrhages, and bad general condition in 55%. In 45% of cases, patients were admitted for intensive chemotherapy, allogeneic stem cell transplantation, or any kind of treatment that requires inpatient care, including certain clinical trials. The median number of hospitaliza-tions per patient was 1 (1-6). 29 patients (13%) died during the year 2005, 59 patients (27%) showed progression to AML. 81% of patients received at least one unit of packed red cells. *Summary*: With regard to MDS subtype distribution, patients seen in our institution did not differ much from the MDS population as a whole. Still, a referral bias is present, reflected by a large proportion of patients requiring inpatient care, either for management of MDS-related complications or intensive treatment of the underlying bone marrow disease.

0943

ACQUIRED α-THALASSEMIA IN MDS (AT-MDS): RARE MUTATIONS DETECTED IN TWO FEMALES

P.S. Haas,[1] M. Schwabe,[1] C.H. Fisher,[2] R. Gibbons,[2] D.R. Higgs,[2] E. Bisse,[1] M. Lübbert[1]

[1]*University of Freiburg Medical Center, Freiburg, Germany;* [2]*John Radcliffe Hospital, Oxford, United Kingdom*

Backgrounds. In contrast to the classical thalassemias, two distinct thalassemias were recently described in which the molecular defect does not reside in the globin genes but in a transcriptional activator of α-globin genes. This protein, dubbed ATRX, is mutated in the rare inherited disease of α-thalassemias (AT) with mental retardation (ATR-X syndrome) whose affected individuals show a mild form of AT. In addition, and independent of the ATR-X syndrome, there have been approximately 100 case reports worldwide of the association of an acquired form of AT with hematological neoplasms, the large majority of those cases being MDS (ATMDS). The clinical characteristics of such patients encompass the typical features of the underlying hematological disorder plus microcytic anemia. The latter is due to massively reduced α-globin gene transcription resulting in excess hemoglobin H (HbH), as revealed by supravital staining of peripheral blood erythrocytes and hemoglobin electrophoresis. The molecular defect of ATMDS lies in a mutation of the ATRX protein and, thus, represents a form of acquired α-thalassemia. ATMDS shows a striking male preponderance. *Methods.* Supravital staining, Southern blotting, and DNA sequencing by denaturing high-performance liquid chromatography. *Results.* Here we report on two female pts with AT-MDS. The first was a 69 year old pt who was diagnosed with MDS-RARS/MPS overlap syndrome (JAK2 negative) with normal karyotype. She presented with microcytic anemia (Hb 7,5 g/dL, MCV 76,5 fl, HbE 22.4 pg) and increasing thrombocytosis (953.000/μL maximum). Supravital staining of a peripheral blood smear revealed erythrocytic HbH inclusions. Sequencing of the ATRX coding sequence revealed a novel missense mutation with an A>G transition in codon 2234. This mutation (D2234G) results in an amino acid substitution in exon 32. It represents the 14th ATRX mutation described thus far and, moreover, the first mutation detected in a female. The second pt (61 years old) with initially RA, normal karyotype (*JAK2* negative) and microcytic anemia (Hb 10.4 g/ld, MCV 69 fl, HbE 15.2 pg) had increasing erythrocytosis of 6.93 Mio/μL maximum. The genetic analysis showed a ATRX point mutation in exon 8 (G521A) which results in an amino acid change from cysteine to tyrosine and consequently in a loss a zinc finger. *Conclusions.* Though AT-MDS is mostly diagnosed in males we have diagnosed two females, both showing ATRX mutations. Microcytic anemia in association with a hematological neoplasm, most commonly MDS, should alert to ATMDS. ATMDS is easily diagnosed by supravital staining of peripheral blood smears. Molecular mechanisms by which mutations cause acquired α-thalassemia probably include epigenetic alterations of DNA methylation and chromatin structure. The remarkable thrombocytosis and erythrocytosis, respectively, in our 2 pts are at least suggestive of other phenotypic abnormalities possibly associated with the acquired ATRX genotypes on the MDS background.

0944

SERIAL DETERMINATION OF FLT3 MUTATIONS IN MDS PATIENTS AT DIAGNOSIS, FOLLOW UP, OR AML TRANSFORMATION: FLT3 ITD/ASP835 MUTATIONS INCIDENCE AND THEIR PROGNOSTIC SIGNIFICANCE

G. Georgiou, V. Karali, C. Zouvelou, E. Kyriakou, M. Dimou, S. Chrisochoou, P. Greka, A. Efthymiou, K. Lilakos, L. Petrikkos, K. Dima, E. Basta, P. Panayiotidis

Laikon Hospital, Athens, Greece

Background. The genetic/molecular alterations that lead to MDS are not fully elucidated. MDS can be considered as pre-leukemia but the precise genetic/molecular events occuring during transition to AML are unknown. *Aim*. The aim of this study was a) to investigate the incidence of *FLT3* mutations (ITD/Asp835) in MDS patients at the time of MDS diagnosis and during disease evolution, b) to analyze if the presence of *FLT3* mutations correlates to AML transformation and c) to investigate the prognostic significance of *FLT3* mutations in MDS patients. *Methods*. Genomic DNA was extracted from bone marrow aspirate smears from 97 patients with MDS (RAEB-t and therapy-related MDS were excluded). All patients had bone marrow smears at presentation and at several time points during their follow up (2-10 samples per patient). Patient DNA was amplified by PCR with specific primers for the detection of FLT3 internal tandem duplication (ITD). ITD positive samples (PCR band(s)>329bp) were cloned and sequenced. Asp 835 point mutations in exon 20 of the *FLT3* gene were detected with PCR followed by digestion with EcoRV of the 195 bp PCR product. Non fully digested products (mutated) were cloned and sequenced (10 plasmids/patient) to verify the existence of Asp 835 mutation. Fisher's exact test and unpaired t-test were used for statistical analysis. Survival curves comparison was done by the log-rank test. For all analyses the p-values <0.05 were considered statistically significant. *Results.* Three of the 97 patients had *FLT3* mutations at presentation: one patient with both ITD and Asp835 (RAEB-BM blasts 16%), one patient with ITD only (RAEB) and one patient with Asp835 only (RAEB). Forty two patients progressed to AML including the three patients that carried *FLT3* mutations at MDS diagnosis. The total incidence of *FLT3* mutations at the time of AML progression was 14.3% (6 out of 42), with 3 additional patients acquiring FLT-*FLT3*3 mutation at the time of progression. In these 3 latter patients, FLT3 mutations were detectable in bone marrow samples 4-6 weeks before overt leukemic transformation. All identified *FLT3* mutations were in frame as shown by sequence analysis and suggest a gain of function mutational event. Patients with *FLT3* mutations had 4.5 times higher risk of transformation to AML compared to patients without mutations (Cox's model application). Survival curves comparison by long-rank test showed a statistical significant difference between MDS patients with FLT3 mutations compared with those without mutations (p=0.0001), as well as between transformed MDS patients with *FLT3* mutations compared with transformed MDS patients without mutations (p=0.01). Two extra patients acquired *FLT3* mutations 12 and 32 months respectively after MDS diagnosis; both these patients died 2 and 6 months respectively, after *FLT3* mutation detection, from infection, before evolution to AML. *Conclusion*. Our study shows that FLT3 mutations seem to be the critical additional genetic event that transforms a minority of MDS to AML; these mutations can be detected before transformation to AML and effective FLT3 inhibitors, when available, might be a potent therapeutic modality for these patients.

0945

ABNORMAL PERIPHERAL BLOOD PROGENITORS ARE CONSISTENTLY OBSERVED AFTER CELL CULTURE IN MYELODYSPLATIC SYNDROME (MDS), ALLOWING THE DIAGNOSIS OF EARLY STEP OF THE DISORDER

I. Dobo, S. Hainos-Godon, F. Geneviève, J. Gérard, M. Gardembas, A. Schmidt-Tanguy, M. Hunault-Berger, M. Zandecki, A. Godon

University Hospital, Angers, France

Background. The diagnosis of MDS may be difficult if dysmyelopoietic and cytogenetic features are absent or inconspicuous, and in many instances follow up with repeated investigations must be achieved to confirm or to rule out the diagnosis. To ascertain whether abnormal progenitors are already present in peripheral blood from MDS patients, and to improve the diagnosis of early steps of the disease. *Methods*. We studied *in vitro* peripheral blood progenitor cell growth from different groups: healthy patients (T)(n=9), MDS (all WHO subtypes)(n=24), non malignant secondary cytopenia(s) (C)(n=9), and one group displaying cytopenia(s) of uncertain diagnosis (n=13). The latter group had follow up for up to 2 years. After percoll (1.077g/mL) separation, blood mononuclear cells (MNC) were collected, $CD34^+$ percentage was determined using flow cytometry, and a number of cells equivalent to 200 $CD34^+$/mL was seeded in semi-solid collagen gel (stem α.4B medium; Stem Alpha, France). After 14 days, collagen gels were transferred to glass slides and stained with MGG to look for colonies growth (number and morphology). *Results*. Median number of colonies (CFU-GM and BFU-E) for 105 MNC was found similar for T (11 and 40) and C (8 and 27), but lower for MDS patients (5 and 8) (p<0.004). Median clonogenic efficiency of $CD34^+$ cells was 3 times lower for CFU-GM and 14 times lower in BFU-E in MDS in comparison with non malignant secondary cytopenias (p<0.001). Morphologic analysis of colonies from collagen gels allowed estimating cellular degeneration: the ratio viable colonies/all colonies (v/a), for both CFU-GM and BFU-E, was always >0.60 in T and C groups, whereas it was always <0.58 in MDS patients (p<0.003). Among the 13 patients displaying cytopenias of uncertain diagnosis, three had a CFU-GM and/or BFU-E v/a ratio<0.58, and 10 a v/a ratio >0.60. The three patients with an abnormal v/a ratio evolved to a MDS (RAEB or RCMD) within 2 years following cell culture. Nine patients with a normal v/a ratio recovered a normal cell count within 6 months (n=6) or developed progressive kidney failure (n=3). One patient with normal v/a ratio remains unclassified after 9 months of follow up. *Conclusions*. Whatever the MDS subtype, cell culture demonstrated that abnormal progenitors were consistently found within peripheral blood, which demonstrated limited *in vitro* growth and a high degeneration rate (apoptosis?). As a high degeneration rate of progenitors was not observed in non malignant disorders, study of peripheral blood progenitors in patients of unknown origin is proposed as a diagnostic tool to ascertain or to rule out diagnosis of early MDS.

0946

INCIDENCE OF MDS WITH 5Q- KARYOTYPE ANOMALIES

U. Germing,[1] C. Strupp,[1] B. Hildebrandt,[1] A. Kuendgen[1], A. Giagounidis,[2] C. Aul,[2] N. Gattermann,[1] R. Haas[1]

[1]*University of Düsseldorf, Düsseldorf, Germany;* [2]*Johannes-Hospital, Duisburg, Germany*

Backgrounds. In a previous study (Germing *et al., Haematologica, 2004*) we found an overall incidence of myelodysplastic syndromes (MDS) of ~5/100.000 per year in the town district of Düsseldorf. The incidence figures were strongly age-related, with a significant rise after the age of 60, particularly in males. We calculated that approximately 4100 new cases of MDS are diagnosed in Germany each year. In individuals below the age of 40 years old, the incidence of MDS is only ~0.4/100.000 per year. *Aims.* The purpose of the present study was to assess the incidence of MDS with cytogenetic aberrations involving 5q-. *Methods.* We looked into the German MDS Registry in Düsseldorf for cytogenetic findings and related them to incidence figures calculated for the town district of Düsseldorf. *Results.* The MDS Registry now comprises 2897 patients, including 2734 with primary MDS (94.4%) and 162 with secondary MDS (5.6%). Median age at the time of diagnosis was 72 years, ranging from 16 to 96. Only 4% of the patients were younger than 40, and 8% younger than 50. There were 1578 males (54.4%) and 1319 females (45.5%). The distribution among IPSS risk groups is as follows: 20% low, 31% intermediate-1, 21% intermediate-2, and 28% high-risk. Accordingly, the low/int-1 group on the one hand, and the int-2/high risk group on the other hand, each have an incidence of about 2.5/100.000 per year, which is similar to that of acute myeloid leukaemia. In 1038 patients (36%), chromosomal analyses were performed at the time of diagnosis. Pa-tients who were karyotyped were significantly younger (median: 64 years) than the MDS pa-tient population as a whole (p=0.0005). Among those with a karyotype available, 180 patients (17%) had a 5q- anomaly, either as a single aberration (n=114) or together with one more chromosomal abnormality (n=12), or as part of a complex karyotype (n=53). This implies an incidence of MDS with 5q- anomalies of about 0.85/100.000 per year, equivalent to nearly 700 newly diagnosed cases per year in Germany. Among patients with a 5q- abnormality in our database, we identified only 21 females younger than 50 yrs and 7 females younger than 40. For males, the figures were similar. To summarize, MDS is one of the most frequent haematological disorders, particularly in the elderly. Patients with karyotype anomalies involving 5q- represent about 17% of MDS pa-tients with an estimated incidence of about 0.85/100.000 per year. Such patients are rare among individuals less than 50 years old. Because of the 'greying of the population' in developed countries, the number of all MDS patients, including those with a 5q- anomaly, is expected to rise.

0947

ALTERATIONS IN THE NATURAL KILLER CELL RECEPTOR REPERTOIRE IN MYELODYSPLASTIC SYNDROMES

B.C. Baumann,[1] M. Jädersten,[2] Y.T. Bryceson,[2] M. Grövdal,[2] N. Björkström,[2] E. Hellström-Lindberg,[2] H.G. Ljunggren,[2] K.J. Malmberg[2]

[1]*Karolinska Institutet, Stockholm, Sweden;* [2]*Karolinska University Hospital Huddinge, Stockholm, Sweden*

Backgrounds. Myelodysplastic Syndromes (MDS) constitute a group of clonal stem cell disorders characterized by ineffective hematopoiesis and pancytopenia. In one third of the MDS patients the disease progresses to acute myeloid leukemia. The only curative treatment for MDS is allogeneic stem cell transplantation (SCT). Several studies have shown that natural killer (NK) cells play an important role in the outcome of SCT in acute myeloid leukemia patients. These results suggest that NK cells may constitute an important therapeutic tool in the treatment of hematological diseases such as MDS. So far, the role of NK cells in the pathogenesis of MDS is poorly understood. *Aim*: The aim of this study was to investigate the NK cell receptor repertoire in patients with MDS. Methods. Bone marrow (BM) and peripheral blood (PB) was collected from patients with MDS and NK cells were analyzed for their receptor repertoire using multi-color flow cytometry. *Results.* MDS patients displayed severe alterations in their NK cell receptor repertoire with decreased expression of several activating NK receptors, including DNAM-1, 2B4, NKG2D, and CD16. These alterations were confined to BM-derived NK cells and did not affect NK cells in PB. One patient had abnormally high levels of $CD56^{bright}$ NK cells displaying a reversed ratio between $CD56^{bright}$ and $CD56^{dim}$ NK cells with 75% and 50% regulatory $CD56^{bright}$ NK cells in BM and PB, respectively. Conclusions. Our preliminary results show that MDS patients display several phenotypic aberrations in their NK cell repertoire. This may have functional consequences and influence pathogenesis and response to immunomodulatory treatments for MDS. Uncovering a role for NK cells in the recognition of MDS tumor cells may set the stage for future NK cell-based immune therapies against MDS.

0948

MYELOID ANTIGEN EXPRESSION ON CD34 POSITIVE BLASTS IN MYELODYSPLASTIC SYNDROMES

B. Leus, W. Renmans, K. Jochmans, H. Schots, I. van de Broeck, F. Trullemans, I. van Riet, M. de Waele

AZ-VUB, Brussels, Belgium

Backgrounds. The myelodysplastic syndromes (MDS) constitute a heterogeneous group of clonal hematopoietic stem cell disorders. They are characterized by abnormal bone marrow (BM) differentiation, peripheral blood cytopenias and a risk of transformation into acute myeloid leukemia (AML). The diagnosis of MDS depends on morphological criteria and cytogenetics and is sometimes difficult to make and subjective. *Aim:* In this study we evaluated the potential of immunophenotyping $CD34^+$ hematopoietic precursors for the diagnosis and classification of myelodysplastic syndromes. *Methods.* Bone marrow samples of patients with different forms of MDS (51 samples), of healthy controls (15 samples), of patients with cytopenia not due to MDS (disease controls; 39 samples) and of patients with AML (25 samples) were examined. MDS

and AML samples were classified according to the WHO criteria. The expression of CD19, CD10, CD133, CD13, CD33, CD117 and CD45 antigens was detected on the CD34+ cells by flow cytometry. Statistical analysis was done with a Mann-Whitney test. *Results.* The number and immunophenotype of the CD34+ cells in BM of disease controls was similar to that in normal bone marrow. Only the number of CD34+ CD133+ positive cells was low. A high number of CD34+ cells was found in MDS and AML. This number correlated with the percentage of blasts found by cytomorphology. The increase of the CD34+ cell number was accompanied by an increase of the myeloid precursors (CD34+ CD117+) and a decrease of the B cell precursors (CD34+ CD19+). CD117 appeared to be the best marker for myeloid precursors, followed by CD13, especially when the number of blasts was high. A wide range of CD34+ CD133+ and of CD34+ CD33+ positive cells was found in all types of samples. CD133 expression was increased in MDS samples with excess of blasts and in AML. No statistical difference was found between the different groups for the CD33 expression. The myeloid antigen expression on CD34+ cells in refractory anaemia (RA), refractory anaemia with ringed sideroblasts (RARS) and refractory cytopenia with multilineage dysplasia (RCMD) was comparable, although a low positivity for CD133 was found in RARS patients. In MDS with excess of blasts, no statistical differences were found between the myeloid antigen expression on the CD34+ cells of the two subtypes (RAEB-1 and RAEB-2). The phenotype of the CD34+ cells in AML patients with less than 30% blasts (previously RAEB-t) was comparable to that found in AML with multilineage dysplasia and other AML patients. *Conclusion*. MDS is characterized by a variable number of CD34+ cells in the bone marrow. This number correlated with the percentage of blasts found by cytomorphology. An increase of the number of CD34+ is accompanied by an increase of the myeloid precursors and a decrease of the B lymphoid precursors. In MDS samples with less than 5% blasts the myeloid antigen expression on the blasts was comparable to that in disease controls. In MDS samples with an excess of blasts the phenotype was closer to that of AML.

0949

LOW-RISK MYELODYSPLASTIC SYNDROMES FROM PIEMONT MDS REGISTRY: A COMPARATIVE REVIEW OF BONE MARROW ASPIRATE SMEARS AND BONE MARROW BIOPSY SPECIMENS

M. Bonferroni,[1] L. Godio,[2] F. Giordano,[2] D. Ferrero,[1] F. Salvi[1], C. Paparo,[3] R. Calvi,[1] M. Grasso,[1] A. Levis,[1] A. Gallamini[1]

[1]*Hematology, CUNEO, Italy;* [2]*Department of Patology, Turin, Italy;* [3]*Laboratory Department, Chieri, Italy*

Aims. to assess the contribute of bone marrow aspirate (BMA) and bone marrow biopsy (BMB) on diagnosis and prognosis of low-risk MDS. Patients and methods. We reviewed 82 cases of MDS with low marrow blasts (≤5%), consecutively admitted in five hospital of Piedmont between 1998 and 2004. All patients were studied on admission, with full blood count, cytogenetics, BMA and BMB. Pts notes were recorded in the archives of Piedmont MDS Registry. Prerequisites for evaluation of morphologic features in MDS included the availability of May Grunwald-Giemsa well stained BMA smears and BMB specimens. BMA were examined for dyserythropoiesis (DE), dysgranulopoiesis (DG) and dysmegakaryopoiesis (DM), as defined by WHO criteria, the percentage of blasts and ringed sideroblasts by a panel of well-trained hematologists. The same was done by two expert pathologists. Inter-observer reproducibility between hematologists and pathologists was estimated. Median survival were calculated by Kaplan-Meyer analysis. *Results.* Median follow up was 27,8 months (0-151). The inter-observer agreement for dysplasia was: good for DE and low for DG, DM and multilineage dysplasia (MuLD). Patients with definite clinical entities such as 5q- syndrome (7 cases), MDS-U (5 cases with thrombocytopenia or neutropenia) and RAEB (8 cases presenting on BMB a percentage of blasts over 5%) were excluded from analysis. 19 cases presented anemia (group A) and 43 pancytopenia (group B). In 14 cases of group A a full concordance was recorded: 4 showed DE and 10 MuLD. By contrast in 5 cases MuLD was recorded in BMA while in BMB only DE was apparent. In group B a full concordance was recorded in 34 cases; 32 showed multilineage dysplasia and 2 only DE. By contrast in 4 cases showing MuLD on BMA, BMB showed DE and 5 cases showing DE on BMA, presented MuLD on BMB. For group A median survival was not reached while group B had a median survival of 40 months (Log Rank test=7.2 $p<05$). Median survival of DE and MuLD were not reached (after 150 months) and 40 months, respectively ($p<0.05$). The same analysis was not statistical significant on aspirate. Conclusions BMB allowed a reclassification in RAEB in about 10% (8/82) of the cases showing a percentage of blasts at the aspirate lower than 5%. BMB has been able to identify more accurately *pure* RA with better prognosis. BMB should be reserved to patients with proven diagnosis of MDS while BMA should be used as first-line, baseline procedure in patients with suspected MDS

0950

OCCURRENCE OF THE JAK2 V617F MUTATION IN PATIENTS WITH REFRACTORY ANEMIA WITH RINGED SIDEROBLASTS ASSOCIATED WITH THROMBOCYTOSIS (RARS-T).

A.F. Remacha,[1] G. Puget,[2] C. Estivill,[1] M.P. Sarda,[1] C. Canals[1], J. Nomdedeu[1]

[1]*Hospital de Sant Pau, Barcelona, Spain;* [2]*Hospital Del Mar, Barcelona, Spain*

Backgrounds. The WHO classification establishes a new category, the Myelodysplastic/Myeloproliferative diseases (MDS/MPD). This category includes myeloid disorders that have both dysplastic and myeloproliferative features. MDS/MPD,U-refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T) is incorporated in this category as a provisional entity. The clinical and morphological features consist of the myelodysplastic syndrome, refractory anemia with ringed sideroblasts (RARS) but with a marked thrombocytosis (>600×10^9/L). The megakaryocytes are enlarged in size. Essential Thrombocytemia (ET) is a Chronic Myeloproliferative Diseases (MPD) . A single point mutation of *JAK2* (Val617Phe) has been detected in half the patients with ET. Aims. The presence of the *JAK2* mutation was assessed in a cohort of patients with RARS, including 3 cases with RARS-T. Methods. We obtained DNA from blood samples from 3 patients with RARS-T. These samples were analyzed using the allele-specific PCR methodology described by Baxter EJ *et al*.[1] DNA samples from 16 RARS and from 21 ET were also studied. Results. In the three cases with RARS-T, the V617F mutation of the *JAK2* gene was detected, but none of the other cases with RARS showed the mutation. Interestingly, endogenous erythroid colony formation *in vitro* was negative in two of them. Bone marrow exams showed hypercellularity with prominent megakaryocytic proliferation, enlarged in size. None of them showed the typical small-sized megakaryocytes of the 5q- syndrome. After a long follow-up (15 years) one case evolved to myelofibrosis . In the ET group, 13 out of 21 cases showed the *JAK2* mutation. *Conclusion*. RARS-T appears to be the coexistence of two disorders, with erythropoiesis showing the characteristics of the RARS and megakaryocytes those of ET. Further data from other groups are necessary to confirm the prevalence of the JAK2 mutation in RARS-T.

References

1. Baxter EJ, et al. Lancet 2005;365:1054-61

0951

WT1 IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES: A USEFUL MOLECULAR MARKER FOR RISK ASSESSMENT

S. Improta,[1] L. Petriccione,[1] M. Sansone,[2] M.R. Villa,[1] M. Sagristani[1], A. Lucania,[1] M. Esposito,[1] C. Tiberi,[2] A. Russo,[2] M.T. Polistina,[2] L. Mastrullo[1]

[1]*PO San Gennaro, Naples, Italy;* [2]*PSI Loreto Crispi, Biologia Molecolare, Naples, Italy*

Backgrounds. Myelodysplastic syndromes (MDS) are clonal hematopoietic stem-cell disorders characterized by ineffective dysplastic hematopoiesis involving one or more cell lineages and characterized by peripheral-blood cytopenias and a high risk of progression to acute myeloid leukemia (AML). According to WHO classification, MDS can be classified in these following groups: refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess of blasts type I and II (RAEB I and II), refractory cytopenia with multilineage dysplasia (RC+Dys), del (5q) sindrome, and MDS unclassifiable (MDS unclass). The Wilms' tumor gene (WT1) is a tumor suppressor gene coding for a zinc-finger transcription factor located on chromosome 11p13, which was originally identified for its involvement in the pathogenesis of the Wilms' tumor. In normal peripheral blood (PB) and bone marrow (BM), WT1 expression is reported to be low and sometimes undetectable even by RT-PCR. By contrast, WT1 is highly expressed in most acute leukemias, and its level of expression is associated with the presence, persistence, or reappearance of leukemic hematopoiesis. *Aims.* WT1 gene expression could represent a useful marker in MDS to establish prognosis and progression of disease. *Methods.* BM samples from 36 MDS patients (16 RA, 7 RAEB I, 4 RAEB II, 4 RARS, 3 deletion of 5q, 2 MDS unclass) were tested for WT1 expression at diagnosis and after 6 months. WT1 gene expression

was evaluated by methods of real-time quantitative PCR (RQ-PCR). *Results*. At diagnosis, 21BM samples (10 RA, 6 RAEB I, 4 RAEB II, 1 RARS, 1 MDS unclass) expressed WT1 transcript amounts greater than the ranges level. The degree of WT1 expression was highly correlated with the type of MDS, was much higher in RAEB I and II compared with RA, and other types, and increased during disease progression. Moreover, a significant correlation was found between WT1 expression levels, blast cell percentage, and the presence of cytogenetic abnormalities. The patients received only a supportive therapy if necessary. After 6 months, 7 patients (2 RA, 3 RAEB I, 2 RAEB II) converted to AML. All of these patients showed at diagnosis an high WT1 expression level and a further elevation of WT1 expression after 6 months. *Conclusion*. WT1 expression has been previously reported to be increased also in myelodysplastic syndromes. In this study, the data obtained show that in most MDS, including a large percentage of RA and almost the total number of RAEB I and II, WT1 is expressed above the range observed in normal controls in BM and that its expression is directly correlated with the type of MDS. In addition, even within each subgroup, a strong association is present between the level of WT1 expression and the blast percentage and the presence of cytogenetic alterations. The identification of a molecular marker so able to establish the tendency of MDS to progression can be of great help in decision making for MDS patients. In conclusion we believe that WT1 can be introduced as a additional marker to the standard parameters already considered in risk assessment for MDS.

0952

COST-EFFECTIVENESS OF CHELATION THERAPY WITH DEFERASIROX VERSUS DEFEROXAMINE IN TRANSFUSION-DEPENDENT MYELODYSPLASTIC SYNDROME

E. Delea,[1] J.F. Baladi,[2] S.K. Thomas,[2] P.D. Phatak[3]

[1]*Policy Analysis Inc. (PAI), Brookline, MA, USA;* [2]*Novartis Pharmaceuticals Corporation, FLORHAM PARK, NJ, USA;* [3]*Rochester General Hospital, Rochester, NY, USA*

Background. Patients with myelodysplastic syndrome (MDS) frequently receive chronic transfusions, along with chelation therapy to prevent complications of iron overload. Deferoxamine is an effective iron chelator, but must be administered as an 8-12 hour infusion 5-7 times per week, leading to poor compliance and/or quality of life. Deferasiroxis a once-daily oral chelator that has been shown to produce reductions in liver iron concentrations and serum ferritin similar to those obtained with deferoxamine. *Aims*. To evaluate from a US perspective the cost-effectiveness of deferasirox versus deferoxamine in patients with transfusion-dependent MDS. *Methods*. Data from a variety of published and unpublished sources were used to estimate the cost-effectiveness of chelation therapy with deferasirox versus deferoxamine in MDS patients receiving frequent transfusions (≥8 per year). As there are no long-term studies describing the complications of iron overload in MDS, we focused on the short-term (i.e., one year) cost and quality-of-life effects of chelation therapy. As comparative data for deferasirox versus deferoxamine in MDS are unavailable, we estimated the average dose of deferasirox based on results for MDS patients in a non-comparative Phase II study (20 mg/kg/d). The relative dose of deferoxamine that would result in similar efficacy (2:1) was based on data from comparative studies in other transfusion-dependent anemias. We conservatively assumed that patients would be fully compliant with chelation therapy. Cost-effectiveness was measured in terms of the ratio of the difference (deferasirox versus deferoxamine) in costs of chelation therapy to the difference in quality-adjusted life years (QALYs) over one year. Unit costs of deferoxamine and deferasirox were based on US wholesale acquisition costs. The cost of deferoxamine administration was based on analyses of health insurance claims data for US patients with transfusion-dependent anemias. Utilities for MDS patients receiving transfusions were based on published data for patients with anemia from metastatic cancer. The difference in quality of life for deferasirox versus deferoxamine was based on a study that used time-trade-off methods to estimate community-based preferences for oral versus infusional chelation. *Results*. One year of treatment with deferasirox is estimated to result in a gain of 0.23 QALYs versus deferoxamine (0.78 versus 0.55). If the price of branded deferoxamine is employed, total annual costs are estimated to be $1,427 greater with deferasirox versus deferoxamine ($45,604 versus $44,177). The cost-effectiveness of deferasirox versus deferoxamine is $6,204 per QALY gained. If the price of generic deferoxamine is employed, costs are increased by $7,025 with deferasirox versus deferoxamine; the cost per QALY gained with deferasirox versus deferoxamine is $30,542. Cost-effectiveness of deferasirox versus deferoxamine was sensitive to the assumed dosages of deferasirox and deferoxamine and the costs and quality of life decrements associated with infusional therapy. *Conclusion*. In patient with transfusion-dependent MDS, the cost per QALY gained with deferasirox versus deferoxamine is well within the range that is generally considered acceptable in the US.

0953

MYELODYSPLASTIC SYNDROMES PRACTICES AND TREATMENT SURVEY

G.J. Mufti

Kings College Hospital, London, United Kingdom

Backgrounds. The Myelodysplastic Syndromes Foundation, Inc., is a non-profit organization established by an international group of physicians and researchers to provide an ongoing exchange of information relating to MDS. *Aims.* A survey of practices and treatments of clinicians with expertise in diagnosing and treating MDS patients was conducted to collect data on current expert clinical management strategies. *Methods.* A 19-question survey developed by hematologists with expertise in MDS was distributed to the MDS Foundation's 102 Centers of Excellence. Survey responses received by email and fax through August 2005 were analyzed using descriptive statistics only. *Results.* Seventy of the MDS Foundation's Centers of Excellence (European, US, and other institutions) responded to the 2004-2005 Practices & Treatment Survey. More than half (56%) of the 32 responding European and other non-US institutions reported higher case loads in the last five years, with 37% attributing the increase to rising participation and interest in clinical trials, 27% to improved diagnostics and treatments, and 16% to increased awareness and referrals. Although 88% of the responding European and other non-US centers indicated that their institution uses the World Health Organization (WHO) classification system, only 25% of referred patients were categorized by WHO subtype. Analyses of treatment strategies among responding European institutions revealed that 41% of patients in Low and Intermediate-1 International Prognostic Scoring System (IPSS) risk groups received supportive care only, 34% received active treatment, and 25% are followed expectantly. For IPSS Intermediate-2 and High risk groups, 29% of patients received supportive care, whereas 67% received active treatment and 5% are being followed. Survey responses showed a wide array of interventions are used for patients of all IPSS risk categories, including transfusions (91%, with 44% indicating transfusion as the only type of supportive care used), erythropoietin alone or with myeloid growth factors, G- or GM-CSF, cyclosporine, antibiotics, vitamins (B12, B6, folic acid), or corticosteroids. Use of erythropoietin was reported as *frequently* by 47% of respondents, *sometimes* by 28%, and *seldom* by 22%. Erythropoietin was reported to be used most commonly in patients with refractory anemia (RA) (88%) and refractory anemia with ringed sideroblasts (RARS) (59%). Median ranking of agents that at the time of the survey were investigative indicated that 5-azacytidine, lenalidomide, decitabine, and oral iron chelation therapy were viewed as the most clinically useful agents; amifostine and calcitriol as least useful; and arsenic trioxide and thalidomide as somewhat useful. *Conclusions.* Survey responses from investigators associated with academic medical centers with clinical research or diagnostic programs in MDS suggest that more disparate MDS treatment approaches may be expected in the office-based setting. The epidemiologic and treatment survey responses indicate that there is an urgent need for establishing guidelines that would assist clinicians in effectively matching patient and disease characteristics with treatment options, particularly as new therapies are emerging and are being evaluated in clinical trials.

0954

DISPARITIES IN CRITERIA FOR INITIATING CHELATION THERAPY FOR IRON OVERLOAD IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS)

P. Fenaux

Hopital Avicenne, University Paris XIII, Bobigny, France

Backgrounds. The Myelodysplastic Syndromes Foundation, Inc., a nonprofit organization established by an international group of physicians and researchers to provide an ongoing exchange of information relating to MDS, conducted an international survey of practices and treatments of clinicians with expertise in diagnosing and treating MDS patients. Survey responses relating to iron overload were studied because it is estimated that more than 40% of MDS patients require regular red blood cell transfusions. *Aims.* To analyze survey data on current expert clinical management strategies for iron overload in MDS. *Methods.* Descriptive statistics were used to analyze The MDS Foundation's 2004-2005 Practices & Treatment Survey responses received by email and fax through August 2005. The survey, developed by hematologists with expertise in MDS, was distributed to the MDS Foundation's Centers of Excellence (48 US and 53 European and other academic medical centers). *Results.* Of the MDS Foundation's 102 Centers of Excellence, 70 (38 US and 32 European and other non-US centers) responded to the survey. Responses indicate that a substantial proportion of MDS patients in all International Prognostic Scoring System (IPSS) risk groups are red blood cell transfusion-dependent: Low risk, 47%; Intermediate-1 risk, 58%; Intermediate-2 risk, 70%; High risk, 82%. Survey responses by European and other non-US centers revealed that an average of 37% of transfusion-dependent patients receive parenteral iron chelation therapy and that the criteria for initiating chelation therapy are not uniform. The number of transfusions was reported as a determining criterion by 47% of respondents, with a mean number of 36 transfusions. 15% of respondents reported that the number of transfusions was their sole criterion for beginning iron chelation therapy. Serum ferritin levels were reported as a determining criterion by 72% of respondents, with the following cutoff values:

Ferritin concentrations for initiating chelation therapy.

>1000 ng/mL	35%*
>1500 ng/mL	17%
>2000 ng/mL	35%
Other (>3000, unspecified)	13%

**% respondents using this cutoff as criterion*

32% of respondents indicated that serum ferritin was the sole criterion used. (97% indicated that they monitored ferritin levels in transfusion-dependent patients irrespective of chelation therapy.) Other criteria used to determine start of chelation therapy included age/life expectancy, MDS subtype, clinical signs of hemochromatosis, quantitative CT liver iron estimation, liver function, transferrin saturation >50%, BMT, anticipated chronic transfusion need, and logistical issues and insurance coverage. A combination of criteria was reported to be used by 38% of respondents. Conclusions. The decision for initiating chelation therapy in transfusion-dependent anemic MDS patients needs to be individualized because of the heterogeneous patient population. However, this data analysis suggests a need for standardizing select criteria, such as the number of transfusions and serum ferritin, for determining when to initiate iron chelation therapy.

Chronic myeloproliferative disorders II

0955

ESSENTIAL THROMBOCYTHEMIA AND PREGNANCY: PRELIMINARY REPORT OF THE PREGNANCY COMMITTEE OF THE REGISTRO ITALIANO TROMBOCITEMIE (RIT)

L. Melillo,[1] A. Tieghi,[2] A. Candoni,[2] R. Ciancia,[3] V. Martinelli,[3] R. Latagliata,[3] G. Specchia,[3] P.R. Scalzulli,[2] R. Fanci,[3] G. Comitini,[2] N. Cascavilla,[2] L. Gugliotta[2]

[1]*Casa Sollievo della Sofferenza IRCCS, S. Giovcanni Rotondo, Italy;* [2]*Hematology Unit, Reggio Emilia, Italy;* [3]*Hematology University, Naples, Italy*

Backgrounds. Essential Thrombocythemia (ET) is diagnosed in the childbearing age in about 20% of patients. Fertility reduction and adverse outcome of pregnancy due to thrombotic or hemorrhagic complications are a matter of concern. *Aim.* To evaluate the outcome of pregnancy in a large series of patients in order to identify a possible guideline for the management of pregnancy in ET. *Materials and Methods.* The pregnancies observed in ET patients in seven Italian Hematological Centres from 1998 to 2005 were registered in the RIT. Results. Fifty-nine pregnancies occurring in 47 women (age 22- 45 years) with ET diagnosed according to the WHO criteria were evaluated. None of these patients had a recognized thrombophilic abnormality other than ET. Besides 37 live births (66%), 7 first trimester losses (12.5%), 6 second trimester losses (10.7%), 2 still births (3.5%) and 5 voluntary abortions on social grounds were described; 3 pregnancies are ongoing. One case of IUGR and 8 premature births at weeks +26, +28, +32, +32, +34, +34, +36 and +36 were reported. Maternal morbidity in this case series was absent. Thirty-six patients (61%) received Aspirin (100 mg) during the pregnancy and 9 out of them also received prophilactic LMWH for six weeks post-partum. Interferon α treatment was performed in 12 patients with a platelet count > $1{,}000\times10^9$/L and considered at high thrombotic risk. The outcome of pregnancies in these 12 patients was the following : 12 live births (70.6%), 2 still births, 2 foetal losses (at weeks +8 and +28) and 1 ongoing. Overall there were 6 premature births at weeks +26, +32 + 33, +34, +34 and +36 respectively. Pregnancy outcome in the remaining group was the following: 22 live births (55%) 11 foetal losses (27.5%), 5 therapeutic abortions and 2 ongoing. Twenty-three pregnancies occurred among 18 women taking Interferon α (10 cases), Hydroxyurea (5 cases), Anagrelide (7 cases) and Busulphan (1 case). The pregnancy had the same outcome than in the overall population : 16 live births (69.5%), 5 foetal losses (21.7%), 1 premature birth (4.3%), 2 therapeutic abortions and 1 ongoing. *Conclusions.* These data confirm that foetal morbidity and mortality is not negligible in ET. Cytoreductive therapy with Interferon α seems potentially able to protect against foetal losses. Although normal pregnancies have been registered in patients who conceived during cytotoxic treatment , the adoption of effective forms of contraception throughout treatment is still strongly recommended. The epidemiological, clinical and biological data on pregnancy in ET obtained by the partecipating Centres are now object of prospective study by the RIT (GIMEMA project) which records the ET patients diagnosed in Italy since January 2004. Therapeutic options including antithrombotic treatment and cytoreductive therapy will be considered and a management plan for pregnancy in ET will be proposed.

0956

EFFICACY AND SAFETY OF PEGYLATED INTERFERON α IN PATIENTS WITH POLYCYTHEMIA VERA A PROSPECTIVE MULTICENTER PHASE II STUDY

K. Merx,[1] A. Fabarius,[2] M. Emig,[2] M. Schatz,[2] H.L. Pahl,[3] B. Stade,[4] H. Kreipe,[5] A. Schmitt-Gräff,[5] J. Thiele,[6] M. Griesshammer,[2] R. Hehlmann,[2] A. Reiter,[2] A. Hochhaus,[2] E. Lengfelder[2]

[1]*Universitätsklinikum Mannheim, Mannheim, Germany;* [2]*Universitätsklinikum Innere Medizin III, Mannheim, Germany;* [3]*Anaesthesiologische Universitätsklinik, Freiburg, Germany;* [4]*Essex Pharma, München, Germany;* [5]*Pathologisches Institut der Universität, Hannover, Germany;* [6]*Institut für Pathologie der Universität, Koln, Germany*

Backgrounds. Interferon α (IFN) is a therapeutic option for patients with polycythemia vera (PV) to control increased myeloproliferation. For pegylated formulations of IFN neither efficacy nor tolerability have been published in larger series of patients (pts) with PV. Aims. A phase II study has been conducted to investigate the antiproliferative effects and the safety of pegylated IFNα2b (PegIntron) in PV. *Methods.* PegIntronα was administered subcutaneously with a starting dose of 50 µg/week. Dose escalation every six weeks to 100 and 150 µg/week or

dose reduction was recommended according to response and toxicity. Pretreatment with one cytoreductive drug but not conventional IFN was permitted in addition to phlebotomy. Complete response (CR) was defined as a stable hematocrit ≤45% without phlebotomy, normalization of platelet counts and normal spleen size. Good partial response (PR1) was defined as reduction of phlebotomies and/or platelet counts and/or splenomegaly >50%, poor partial response (PR2) is the respective reduction between 25-50%. *Results.* Since February 2003, 49 pts (29 m / 20 f) with PV according to the WHO criteria have been enrolled. 21/23 pts investigated were positive for the *JAK2(V617F)* mutation. Follow up data are presently available from 37 pts with a median age of 59 (41-78) years and a median duration of therapy of 23 (2-36) months. At their most recent presentation, two pts received 25 μg PegIntronα/week, 14 pts 50 μg/week, 6 pts 75μg/week, 7 pts 100 μg/week and 8 pts 150 μg/week. A significant reduction of the initial platelet counts ($p< 0.0001$) was already seen after six weeks of therapy. By now, one patient achieved CR, 30 pts achieved PR1. Of these, 13 pts (35%) became free of phlebotomies for at least 6 months and achieved a normalization of platelet counts. However, normalization of spleen size has not been reached so far. Five pts achieved PR2, one patient had no change and another patient progressed to myelofibrosis after initial PR1. Side effects WHO grade I-II were observed in all 37 pts (arthralgia and flu-like symptoms in 84%, fatigue in 75%, injection site reactions in 57%, gastrointestinal symptoms in 51%, hepatotoxicity in 35%, depression in 32%, exanthema in 21%, alopecia in 16%, sexual dysfunction in 11% and hematotoxicity in 5%). WHO grade III depression or fatigue and arthralgia was observed in one patient each. Dose reduction due to side effects was necessary in 15 pts (40%). PegIntronα was withdrawn in 12 pts (32%) after a median duration of therapy of 14 (2-28) months due to toxicity or patient's refusal to continue therapy (n=9), progression of the disease (n=1) or PV-related complications (n=2). In 12 out of 28 investigated pts a decrease of the PRV-1 expression during therapy was observed. Retrospective mutation screening for JAK2(V617F) will be performed in all patient samples available prior to therapy and during follow-up. Conclusion: PegIntronα is an effective cytoreductive therapy for patients with PV. The reduction of the elevated platelet counts indicates the sensitivity to the drug in the early phase of therapy.

0957

RELATION BETWEEN PROPORTION OF GRANULOCYTE JAK2 (V617F) MUTANT ALLELES, CLINICAL PHENOTYPE AND DISEASE PROGRESSION IN CHRONIC MYELOPROLIFERATIVE DISORDERS

F. Passamonti,[1] E. Rumi,[2] D. Pietra,[2] S. Boggi, C. Elena,[1] M.G. Della Porta,[1] L. Arcaini,[2] E. Boveri,[2] C. Pascutto,[2] M. Bonfichi,[2] M. Lazzarino,[1] M. Cazzola[1]

[1]*University of Pavia, Pavia, Italy;* [2]*IRCCS Policlinico San Matteo, Pavia, Italy*

Background. The occurrence of the somatic gain-of-function *JAK2* (V617F) mutation in a hematopoietic stem cell can result in selective expansion and activation of its myeloid-lineage cell progeny, and consequently in a myeloproliferative disorder. By using sensitive assays, the mutation is found in most patients polycythemia vera (PV) and in about half of those with essential thrombocythemia (ET) or chronic idiopathic myelofibrosis (CIMF). *Aims.* Since the proportion of mutant alleles in positive cases may range from about 1% to 100% (Passamonti et al, Blood 2005 Dec 22; Epub ahead of print), we investigated the biological and clinical significance of this apparently heterogeneous involvement. *Methods.* We used a quantitative real-time polymerase chain reaction (qRT-PCR)–based allelic discrimination assay for the evaluation of granulocyte *JAK2* (V617F) mutation status in 419 patients diagnosed with a myeloproliferative disorder. *Results. JAK2* (V617F) was detected in 133/150 (89%) patients with PV, 62/125 (50%) patients with ET, and 55/91 (60%) patients with CIMF; in addition, it was found in 31/31 (100%) patients with post-PV MF and in 13/22 (59%) patients with post-ET MF. Patients with PV had higher percentages of *JAK2* mutant alleles than those with ET, and patients with fibrotic CIMF had higher values than those with prefibrotic CIMF; patients with post-PV myelofibrosis had the highest percentages of mutant alleles. Overall, the longer the time elapsed from diagnosis, the higher the percentage of mutant alleles; sequential studies in a subgroup of patients showed that on average the proportion of mutant alleles increased with time. Granulocyte *JAK2* mutant alleles were greater than 50% in all patients with 9pLOH studied, and lower than 60% in all individuals without 9pLOH. Studies of circulating erythroid progenitors showed a relationship between colony JAK2 (V617F) mutation status (fully wild-type, heterozygous or homozygous colonies) and the percentage of mutant alleles in circulating granulocytes. We grouped positive patients with PV, ET and post-PV MF, and studied the relationship between percentage of *JAK2* mutant alleles and clinical phenotype. Median Hb was higher in patients with 25%-75% than in those with less than 25% of mutant alleles. By contrast, the highest median value for PLT count was found in this latter subgroup, and PLT counts decreased with increasing proportion of mutant alleles. Interestingly, the opposite was true for both WBC count and spleen size. Similar observations were made when positive patients with CIMF were analyzed according to their proportion of granulocyte *JAK2* (V617F) alleles. *Conclusions.* As regards pathophysiology, our findings are consistent with a two-step model of clonal evolution of myeloproliferative disorders involving a transition from heterozygosity to homozygosity for the *JAK2* (V617F) mutation in hematopoietic cells. From a clinical viewpoint, the present observations suggest that low proportions of mutant alleles (< 25%) are mainly associated with thrombocytosis, intermediate proportions (25-75%) with erythrocytosis, and high proportions (>75%) with myeloid metaplasia and splenomegaly. Physiological and genetic modifiers are expected to further influence the clinical phenotype.

0958

HOMOZYGOSITY FOR JAK2V617F IDENTIFIES MPD PATIENTS WITH A MORE SYMPTOMATIC DISEASE. AN ITALIAN GIMEMA RETROSPECTIVE STUDY ON 989 PATIENTS

M. Vannucchi,[1] G. Barosi,[2] A. Rambaldi,[3] R. Marchioli,[4] T. Barbui,[3] for the GIMEMA

[1]*University of Florence, Florence, Italy;* [2]*IRCCS Policlinico S.Matteo, Pavia, Italy;* [3]*Ospedali Riuniti, Bergamo, Italy;* [4]*Consorzio Mario Negri Sud, Chieti, Italy*

Background. An acquired mutation in the *JAK2* gene is found at different rates in patients with chronic myeloproliferative disorders (MPD); in about 20% of polycythemia vera (PV) or idiopathic myelofibrosis (IM), and in less than 3% of essential thrombocythemia (ET), the mutation is harboured in the homozygote status. This low frequency of homozigosity has prevented until now the elucidation of its impact on disease phenotype. *Aims.* The aim was to evaluate whether homozygosity for JAK2V617F pointed to a subgroup of MPD patients with unique clinical characteristics. *Design and Methods*. In an Italian cooperative GIMEMA retrospective study, 989 MPD patients were enrolled from 11 hematology centers. The diagnosis of PV was made in 328 (33%), of ET in 400 (40%), while 224 (23%) were IM and 37 (4%) were post-PV/ET forms (PP/PTMM) of myelofibrosis. Diagnosis of PV or ET was made accordingly to either the PVSG or WHO criteria, while the Consensus Conference Criteria were used for IM. The only eligibility criteria for inclusion was the availability of a *JAK2V617F* mutational status determination according to the ASO-PCR and the BsaXI digestion method (Baxter, Lancet 2005). *Results*. 317 patients (32%) were wild-type (WT), 520 (53%) were *JAK2V617F* heterozygote and 152 (15%) homozygote; among the latter, 81 were PV, 8 ET, 45 IM and 18 PP/PTMM, accounting for 25%, 2%, 20%, and 49%, respectively, of patients within each diagnostic group. Irrespective of their diagnosis, homozygote patients showed higher leukocyte count and hematocrit level, while platelets were unchanged. The frequency of splenomegaly progressively increased from 40%, to 51% to 69% in WT, heterozygotes or homozygotes; similarly, the occurrence of pruritus rose from 8% to 18% to 28%, and that of systemic symptoms from 25% to 30% to 38%. There were 351 thrombotic events, of which 251 were major events and 187 of the microvessels; major hemorrhages were 45. There was a higher incidence of thrombosis in homozygotes (55%) than in heterozygotes (36%) or WT (26%), while there was no difference in hemorrhages. In PV and ET, homozygosity was associated with a greater risk of evolution into myelofibrosis (12% and 25%, respectively) compared to heterozygosity (2.2% and 2.5%); noteworthy, the highest frequency of homozygosity was recorded among PP/PTMM patients (49%). Finally, the frequency of patients overexpressing PRV-1 gene was greater among homozygotes (89%) than heterozygotes (69%) or WT (42%). *Conclusions*. This large survey of MPD patients, that allowed to evaluate 152 homozygote patients, supports the contention that the loss of wild-type JAK2 allele in hematopoietic cells characterizes a quite homogeneous category of patients with more symptomatic disease within each MPD diagnostic category. Assessment of JAK2V617F homozigosity may have a role in risk prediction and patient management.

GIMEMA MPD WP: Antonioli E, Guglielmelli P, Longo G, Bosi A (Firenze); Marchetti M (Pavia); DeStefano V, Leone G (Roma); Alimena G, Foà R (Roma); Ruggeri M, Rodeghiero F (Vicenza); Specchia G, Liso V (Bari); Gerli G, Cattaneo M (Milano); Piazza R, Pogliani E (Monza); Carraro MC, Rossi E (Milano); Tieghi A, Gugliotta L (Reggio Emilia); Marfisi MR (Chieti).

0959

WHAT IS THE BEST STRATEGY FOR THE DIAGNOSIS OF POLYCYTHEMIA VERA?

F. Girodon,[1] P. Mossuz,[2] E. Lippert,[2] N. Boiret,[2] I. Dobo,[2] J.F. Leseves,[2] V. Praloran,[2] M. Donnard,[2] S. Hermouet[2]

[1]*Laboratoire d'hmatologie, Dijon, France;* [2]*CHU, Grenoble, France*

Background. The diagnosis of polycythemia vera (PV) is based on major and minor, biological and clinical criteria (World Health Organisation or the Polycythemia Vera Study Group). These classifications do not use the recently described *JAK2-V617F* mutation as a criteria. Because of its high frequency in PV, and to a lesser degree, in other myeloproliferative disorders (MPD), the place of this new MPD marker among other biological tests has to be defined and validated. In most of the published reports on the frequency of the *JAK2-V617F* mutation in PV, patients were not at diagnosis. *Methods.* We report here the specificity and sensibility of different approaches in the diagnosis of PV in a large cohort of 421 patients suspect of polycythemia; the *JAK2-V617F* mutation was studied in 124 patients. *Results*. Four hundred and twenty one patients (307 males, 114 females) were examined for persistent elevation of haematocrit (Hct). The RCM was measured in 365 patients and absolute erythrocytosis was confirmed for 290 patients. A high Hct (≥56% in women and ≥60% in men) was always associated with high RCM (> 125%) and was observed in 27% of patients. Depending on the criteria used, 208 patients (WHO criteria) or 167 patients (PVSG criteria) were diagnosed with PV. Thirty-two out of the 41 patients not diagnosed as PV following the PVSG criteria had endogenous erythroid colonies (EEC), a major WHO criterion. Altogether we observed 72 apparent erythrocytosis (AE), ie. patients with a RCM < 125%, 64 secondary erythrocytosis (SE) and 77 idiopathic erythrocytosis (IE). Following two diagnostic approaches recently proposed (*A Tefferi and JL Spivak, seminars in Hematology, 42:206-220, 2005*), one based on RCM and oxygen saturation prior to the detection of *JAK2-V617F*, the other on serum EPO followed by the detection of *JAK2-V617F* and bone marrow biopsy, many patients were misdiagnosed: 6% using the first approach, 2% using the second one. In term of expense, the first approach requests to test the RCM and the *JAK2-V617F* mutation in 73% and 45% of patients, respectively, whereas it is necessary to perform a bone marrow biopsy and the detection of the *JAK2-V617F* mutation in 54% and 96% with the second approach.The patients who presented a high Hct (>54% in males and >52% in females) and a thrombocytosis (>400 10^9/l) were all diagnosed as PV according to the WHO classification (n=81). Those with a high serum epo were all diagnosed as not PV (n=22). The RCM was reserved for the patients without the JAK2-V617F mutation. Conclusion : We propose a new approach, based on the JAK2 mutation as a first intention test associated with the blood cell count and the serum epo. The RCM should be reserved in *JAK2-V617F* negative patients.

0960

PREVALENCE OF THE JAK2 V617F MUTATION IN PATIENTS WITH SPLANCHNIC OR CEREBRAL VENOUS THROMBOSIS AND WITHOUT SIGNS OF OVERT CHRONIC MYELOPROLIFERATIVE DISORDER

V. De Stefano,[1] A. Fiorini,[2] E. Rossi,[2] T. Za,[2] G. Farina,[2] G. Reddiconto,[2] P. Chiusolo,[2] S. Sica,[2] G. Leone[2]

[1]*Catholic University, Rome Italy;* [2]*Inst. Hematology, Catholic University, Rome, Italy*

Backgrounds. Thrombosis of splanchnic or cerebral veins can develop in patients with chronic myeloproliferative disoders (CMD) such as polycythemia vera (PV) and essential thrombocythemia (ET); a CMD at very early stages not fulfilling diagnostic conventional criteria can account for a substantial proportion of all observed splanchnic venous thrombosis. Recently a somatic mutation (V617F) of the Janus kinase 2 (JAK2) was reported in in a high proportion of patients with chronic myeloproliferative disorders (CMD). No data are available on the presence of the JAK2 mutation in patients with thrombosis of splanchnic or cerebral veins. *Aims.* To estimate the prevalence of the V617F JAK2 mutation in patients with thrombosis of hepatic/portal/mesenteric/splenic veins or cerebral veins in the absence of conventional criteria for diagnosis of CMD. *Methods.* We studied 111 adult patients (M/F 45/66, median age 40 years, range 18-79) with venous thrombosis of unusual sites: 12 with hepatic vein thrombosis (HVT), 60 with portal-mesenteric vein thrombosis (PMVT), and 39 with cerebral vein thrombosis (CVT). No patient fulfilled conventional criteria for diagnosis of PV or ET. For comparative purpose 19 patients with overt CMD (6 with PV, 12 with ET, and 1 with idiopathic myelofibrosis IMF) were also investigated (M/F 8/11, median age 33 years, range 21-80); clinical manifestation was HVT in 3 patients, PMVT in 12, and CVT in 4. All patients were screened for the presence of the *JAK2* mutation and thrombophilia (deficiency of antithrombin, protein C or S, factor V Leiden, prothrombin G20210A, hyperhomocysteinemia, lupus anticoagulant, anticardiolipin antibodies, anti-β2-glycoprotein antibodies). *Results.* The *V617F* JAK2 mutation was found in 17 (89.4%, 95% CI 68.6-97.0) of the 19 patients with overt CMD and thrombosis of unusual sites. In 9 cases (47.3%, 95% CI 27.3-68.2) the JAK 2 mutation was the only putative risk factor, in the absence of thrombophilia or any circumstantial risk factor (oral contraceptives, surgery, puerperium, trauma). In the 111 patients without overt CMD a thrombophilic alteration was present in 36.9% (95% CI 28.5-46.2) of the cases. The *JAK2* mutation was found in 13 (18.0%, 95% CI 10.8-28.4) of the 72 patients with HVT or PMVT and in 2 (5.1%, 95% CI 1.4-16.8) of the 39 patients with CVT. No difference was found in the prevalence of JAK2 mutation between patients with HVT and those with PMVT (p= 1.0). In 7 cases with HVT or PVMT (9.7%, 95% CI 4.7-18.7) without diagnosis of overt CMD the JAK2 mutation was the only putative risk factor. *Conclusions.* The *V617F JAK2* mutation was detected in the large majority of the patients with overt CMD and splanchnic or cerebral venous thrombosis; in the absence of overt signs of CMD the mutation is still present also in a substantial proportion (18%) of patients with splanchnic venous thrombosis and in a minority (5%) of patients with cerebral venous thrombosis. Screening for the V617F JAK2 mutation in such patients could identify CMDs at very early stages, having thrombosis as heralding symptom.

0961

ASSAY OF THE STROMAL CELLS IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISEASES

L.P. Ludmila, G.Y.U. Miterev, K.S. Momotjuk, M.V. Vakhroucheva, N.V. Tzvetaeva, M.S. Sokolova, A. Turkina, N.D. Khoroshko

National Hematology Research Centre, MOSCOW, Russian Federation

Background. Chronic myeloproliferative diseases (CMD) is a group of malignant clonal disorders of haemopoietic stem cells. Malignant haemopoietic cells may exert influence on the stromal cells, which may result in alteration of the haemopoietic microinvironment. *Aims*. The purpose of the present study was to estimate functional activity of the hemopoietic microenvironment in CMD patients. We have studied the ability of bone marrow stromal cells from CMD patients to support proliferation and differentiation of cobblestone areas forming cells (CAFC) of normal individuals. *Methods*. Stromal and hematopoietic cells were obtained from bone marrow aspirates of 9 CMD patients (4 patients with idiopathic myelofibrosis, IMF, and 5 patients with chronic myeloid leukemia, CML) and 6 normal individuals. We used the stromal feeder layers after irradiation (48 Gy): 3-4 week long-term cultures (LTC) and fibroblasts stimulated to osteogenic differentiation by dexamethasone (10^(-7)M). The normal haemopoietic cells were seeded on the stromal cells of the CMD patients and of normal individuals. Functional activity of stromal cells of CMD patients was defined by the number of cobblestone areas-forming cells (CAFC) on different stromal feeder layers in LTC bone marrow by limiting dilution assay. Stromal cells of the normal individuals were used as a control. The late (1-5 weeks incubation) and early (6-9 weeks incubation) CAFCs were estimated. *Results.* Our results showed that all examined stromal cells from IMF and CML patients supported growth of the CAFCs of the normal individuals up to 9 week incubation. We have detected that number of the early (0,6±0,3) and late (4,8±0,7) CAFCs was decreased on stromal layers of LTC of the IMF patients in comparison with the normal stromal layers of LTC (early - 7,5±1,1 and late -45,3±6,1) CAFCs. The number of the early CAFCs (4,4±1,7) was reduced on IMF patient fibroblasts stimulated to osteogenic differentiation too, whereas the number of the early CAFCs on the stimulated normal fibroblasts was 10,2±1,9. The number of the early and late CAFCs in the CML patients was as on the normal stromal cells. Conclusion. IMF patient stromal cells can not support growth of the normal CAFCs, suggesting that the haematopoietic microinvironment have a functional defect in IMF patients.

0962

GENE EXPRESSION PROFILING IN ESSENTIAL THROMBOCYTHEMIA USING CDNA MICROARRAY TECHNOLOGY. PRELIMINARY RESULTS

E. Puigdecanet,[1] B. Espinet,[1] B. Bellosillo,[1] J. Lozano,[2] E. Gimeno[1], E. Abella,[1] A. Salar,[1] L. Sumoy,[2] F. Solé,[1] C. Besses,[1] S. Serrano[1], L. Florensa[1]

[1]*Hospital del Mar, Barcelona, Spain;* [2]*Centre de Regulaciò Genòmica, Barcelona, Spain*

Background. Essential thrombocythemia (ET) is a chronic myeloproliferative disorder (CMPD) lacking specific molecular markers. Consequently, its diagnosis is based on exclusion of other CMPD and secondary thrombocytosis. *Aim*. The aim of the study was to characterize the gene expression profiling in ET using cDNA microarray technology, specially analyzing the implication of the JAK-STAT signaling pathway. *Patients and Methods*. Peripheral blood granulocytes obtained from 20 ET patients diagnosed according to the PVSG criteria (1997), who had not received platelet-lowering therapy, were isolated. Good quality RNA (RIN>7) was hybridized competitively to the RNA granulocytes obtained from ten pooled healthy individuals. Duplicates were performed with dye-swap to control for possible differences in the incorporation rate of the two fluorochromes. Oligonucleotide cDNA microarray expression profilings were obtained using 44K whole human genome oligo microarrays (Agilent Technologies, Palo Alto, CA), comprising 41.000 oligonucleotides. Fluorescent images were obtained using an Agilent G2565BA scanner and Genepix 6.0 (Axon Inc.) was used to extract data from the image and analysis was performed using the R-package. *Results*. From 41.000 oligonucleotides covered in the Agilent platform, an homogenous TE signature was obtained in 17 out of 20 patients studied. This signature was composed of 124 genes that were found to be more than 2-fold up-regulated and 14 genes 2-fold down-regulated, in relation to control granulocytes. The other 3 patients showed a distinct expression profile and different to each other. Of the 124 up-regulated genes, 101 had an assigned function, being the immune response the most implicated, with 29 genes overexpressed. In addition, cellular movement (23 genes) and hematological system development and function (28 genes) were also involved. The most important involved network comprised 35 genes, mainly *CXCL2, CD83, PTGS2,CCL3L1, GCH1* and *TNFAIP3*, which mediate immune and inflamatory responses. Two other networks also implicated included cellular growth and proliferation, cellular movement and hematological system development and function (13 genes, being the most important *DUSP2, ETS2, GADD45D*) and cancer, cell cycle, and cell morphology (13 genes, among them DNAJH1). An interesting group of up-regulated genes was the chemokine family, involved in cell movement, chemotaxis, signal transduction and cell communication (*CXCL2, CCL4, CCL3, CCL20, CCL23*). Regarding to the 14 down-regulated genes, 9 of them had an assigned function and included transcription factors (SOX4, ZNF217), genes involved in immune response (C4BPA, C1QG), cellular protein metabolism (*TBCC, SOLH*), cellular localization and intracellular transport (AP3M1). PRV-1, c-MPL and TPO gene expression was not affected in any of the 20 patients studied. Interestingly, only one gene (CXCL2) involved in the JAK-STAT signaling pathway was affected. No differences regarding expression patterns were found between ET patients with and without the *JAK2 V617F* mutation. *Comments*. Our preliminary results have shown an homogeneous expression pattern in 17/20 ET patients. It is remarkable that an important number of genes were up-regulated, most of them being implicated in the immune response, cellular movement and hematological system development and function.

Funding. Grants FIS PI030345, C03/07 and C03/10 from the Spanish Ministry of Health.

0963

DEVELOPMENT AND CLINICAL CORRELATES OF A QUANTITATIVE ARMS ASSAY FOR V617F MUTATED JAK2 RNA IN CHRONIC MYELOPROLIFERATIVE DISORDERS

P. Guglielmelli,[1] A. Pancrazzi,[2] E. Antonioli,[2] V. Ponziani,[2] S. Mappa,[2] C. Bogani,[2] L. Pieri,[2] G. Poli,[2] A. Bosi,[2] A.M. Vannucchi[2]

[1]*Az. Ospedaliero Universitaria Careggi, Florence, Italy;* [2]*Azienda Ospedaliero Universitaria Careggi, Florence, Italy*

Background. A point mutation in the JAK2 gene has been described in patients with chronic myeloproliferative disorders (MPD), but the clinical significance of JAK2V617F, that may be harboured in either the heterozygote or homozyote status, is still largely undefinied. There are indirect suggestions that clinical phenotype, as well as some biological characteristics, are dependent on the mutated allele levels; however, information about quantitative allele determination is scarse to date. *Aims*. The aim of this work was to develop a quantitative assay for mutated *JAK2 RNA* that might potentially provide novel information about the role of the mutation in determining disease phenotype. *Methods*. We have designed and validated in 179 MPD patients an amplification-refractory mutation sequencing (ARMS) PCR assay that allows the relative quantitation of mutated and normal JAK2 mRNAs using dye-labelled mutation-specific primers; capillary electrophoresis was used to resolve the two amplicons of 224 and 199 bp, corresponding to WT and mutated allele respectively, and the peak area ratio of the two amplicons was then calculated. *Results*. Time course studies demonstrated that the assay is reproducible even when blood processing is delayed up to 24 to 36 hours after sampling. Direct sequencing confirmed the expected sequence of the two generated amplicons. Curve dilution experiments showed a detection limit for the assay of about 1%; 50 healthy subjects always resulted WT, while the HEL cell line (known to be JAK2 V617F homozygote) showed 100% mutated RNA. The greater sensitivity of this assay allowed to identify 9% more JAK2-mutated patients as compared to conventional allele-specific (ASO) and BsaXI digestion PCR (from 116 to 132 JAK2 mutated patients). The mutated mRNA ratio ranged from 5% to 51% in the *JAK2V617F* heterozygote (according to the BsaXI assay) and from 45% to 100% in the homozygote patients. Patients with essential thrombocythemia (ET) showed significantly lower JAK2 mutated RNA levels (median, 13.2%) than either polycythemia vera (PV; median 63%) or idiopathic myelofibrosis (IM; median, 68.3%) patients ($p<0.01$). Considering all patients together, we found a statistically significant correlation between the amount of mutated JAK2 mRNA and the expression levels (by RealTime PCR) of either PRV-1 or NF-E2 gene, previously found to be overexpressed in MPD patients. However, there were also differences according to the diagnosis: in fact, significant correlations between percentages of mutated *JAK2 RNA* and PRV-1 and/or NF-E2 RNA levels could be ascertained in PV and IM patients (in both instances with a p at least <0.01), but not in ET. *Conclusions*. The ARMS technique described herein seems to have some advantages over available genomic techniques due to a greater sensitivity, but most importantly it provides a reliable estimate of the levels of wild-type and mutated *JAK2 RNA* in the cells; the potential clinical impact of this quantitative approach is supported by the observation that abnormal expression of PRV-1 and/or NF-E2 was dose-dependent with the levels of mutated *JAK2 RNA* in granulocytes, and might prospectively lead to a better clinically-oriented assessment of the impact of *JAK2V617F* mutation in MPD.

0964

MOLECULAR ANALYSES IN FAMILIAL AND SPORADIC CONGENITAL PRIMARY ERYTHROCYTOSIS

H. Cario,[1] S. Rives Sola,[2] A. Neususess,[3] E. Kohne,[1] K. Schwarz[4]

[1]*University of Ulm, Ulm, Germany;* [2]*Hospital Sant Joan de Du, Barcelona, Spain;* [3]*University Hospital Schleswig-Holstein, Kiel, Germany;* [4]*Institute of Clin. Transf. Medicine, Ulm, Germany*

Backgrounds. The only molecularly characterised type of primary familial and congenital erythrocytosis/polycythemia (PFCP) is caused by dominant mutations in the erythropoietin-receptor gene (EPOR). EPOR mutations are estimated to account for 12-15% of cases with congenital primary erythrocytosis. So far, at least fourteen different EPOR mutations have been described, eleven of them leading to a truncation of the intracellular part of the receptor, resulting in hypersensitivity of erythrocyte progenitors to circulating Epo. The majority of previously reported mutations has not been identified in additional patients outside of the index family. *Aims.* Search for the underlying genetic cause in patients with familial or sporadic congenital primary erythrocytosis of so far unexplored origin: 1. Analysis of EPOR to identify unknown mutations or any of the previously reported mutations if occurring independently of the original family. 2. In patients without EPOR changes, exclusion of a somatic or genomic *JAK2 V617F* mutation which was previously detected in patients with polycythemia vera (PV) and other myeloproliferative disorders. Method: 16 patients (age range 5-66 years) with a serum Epo level of < 10 mU/mL have been included in this study, 3 of them being related (a mother and two of her sons). *P. vera* was excluded according to PVSG or WHO diagnostic criteria. Sequencing analysis of coding regions and intron/exon boundaries of the *EPOR* gene was performed on genomic DNA. An allele-specific PCR was used to exclude or diagnose the JAK2 V617F mutation. *Results.* An EPOR mutation 1453G→A creating a termination signal at codon 439 (Trp439ter) was

found in a 5 year old Spanish girl. Her parents and her brother do not present this mutation and have normal blood counts. Another EPOR mutation (EPOR 1414C→G, Tyr426ter) was detected in the multimember family case . Interestingly, the mother currently presents with normal hemoglobin and hematocrit levels (only mild microcytosis). She had been included in the study because of her affected sons but also because of her documented erythrocytosis during childhood and adolescence. This change of the clinical presentation is of particular interest since the original identification of this mutation in first PFCP family had been complicated by the fact that one family member with the mutation was apparently clinically unaffected. None of the patients presented the *JAK2 V617F* mutation. *Conclusion*: EPOR gene mutations causing either sporadic or familial primary congenital erythrocytosis are found in patients of various ethnic origins. Considering the fact that 3 of the 16 patients were from one family, the previously reported prevalence of EPOR gene mutations in the group of primary erythrocytosis of about 15% is confirmed by this study. The mutation EPOR 1414C→G previously described was independently detected in a second family and is associated with a variable phenotype. The exploration of the underlying pathophysiological mechanisms may contribute essentially to the knowledge about erythropoiesis' regulation. The PV-characteristic mutation *JAK2 V617F* does not seem to play a role in congenital primary erythrocytoses.

0965

THE LEVELS OF JAK2V617F RNA DICTATE THE CLINICAL PHENOTYPE IN POLYCYTHEMIA VERA AND IDENTIFIES PATIENTS WITH MORE SYMPTOMATIC DISEASE

E. Antonioli, P. Guglielmelli, S. Mappa, A. Pancrazzi, C. Bogani, V. Ponziani, L. Pieri, G. Longo, A. Bosi, A.M. Vannucchi

Az Ospedaliero-Univarsitaria Careggi, Florence, Italy

Background. The occurrence of an unique *JAK2V617F* mutation in phenotypically distinct chronic myeloproliferative disorders (MPD), including polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IM), suggests that other genetic events/gene modifiers might be involved. *Aims*. As an approach to unravel significant associations between phenotype and the *JAK2* mutation, we have correlated the levels of *JAK2V617F RNA* with clinical and laboratory characteristics at the diagnosis in 63 patients with polycythemia vera (PV) and 115 with essential thrombocythemia (ET), as diagnosed according to the WHO criteria. *Methods*. Wild-type and mutated JAK2 RNA levels were determined by an amplification-refractory mutation sequencing (ARMS) PCR assay on granulocytes, and expressed as the percentage of mutated *JAK2 RNA* over total (*Vannucchi AM et al, Leukemia, In press*). *Results*. 53/63 PV patients (84%) and 76/115 (66%) presented detectable levels of *JAK2V617F RNA*; the amount of mutated RNA was higher in PV than in ET granulocytes (median 52% and 12.5%, respectively; $p<0.0001$). In PV patients, the hematocrit and white blood cell count were significantly related to the amount of mutated RNA, while there was an inverse relationships with MCV and platelet count. None of these parameters significantly correlated with mutated RNA levels in ET. Even when the analyses were restricted to those PV patients who showed RNA levels in a range similar to that observed in ET (1-55%) the above correlations were mantained, thus ruling out that these effects might be simply ascribable to the overall higher load of JAK2V617F RNA in PV than in ET patients. Among PV patients with JAK2V617F mutation, the frequency of splenomegaly, of therapy (flebotomies and chemotherapy) and of chemotherapy requirement were all significantly increased over wild-type patients, but again not in ET pts. On the other hand, in both PV and *ET JAK2V617F* mutated patients there was a greater frequency of EEC and overexpressed PRV1 gene, while there was no difference in $CD34^+$ cell count in the peripheral blood. The percentage of *high-risk* patients was higher among mutated than wild-type ones (63% vs 27%, $p=0.003$) if patients were all considered together, but did not reach the significance level in the ET group alone (62% vs 38%, $p=0.07$); on the contrary, in PV there was a progressive increase in the percentage of high-risk patients according to the amount of mutated RNA (10% in wild-type, 24% in patients with 1-25% *JAK2V617F RNA* and 66% among those showing 26-100% *JAK2V617F RNA*). *Conclusions*. By quantifying the amount of *JAK2V617F RNA* in granulocytes, we documented a gene dosage-effect in PV, but not in ET, suggesting that the *JAK2* mutation dictates the clinical phenotype in PV patients while additional genetic or host factors modulate the disease presentation in ET. Also of note, the levels of *JAK2V617F RNA* identified PV patients with more symptomatic disease in terms of blood abnormalities, therapy requirement and *high-risk* category.

0966

DIAGNOSIS OF ESSENTIAL THROMBOCYTHEMIA: THE USEFULNESS OF JAK2 V617F MUTATION DETECTION IN PLATELETS RNA

O.A. Calendini,[1] C. Marzac,[1] I. Teyssandier,[1] A. Vandamme,[2] F. Delhommeau,[3] O. Legrand,[4] N. Casadevall[3]

[1]*Hopital Htel-Dieu, Paris, France;* [2]*Hopital Henri Mondor, Labo., Creteil, France;* [3]*INSERM U*^{56}*2, institut Gustave Roussy, Villejuif, France;* [4]*Hematologie Clinique, Hopital Htel-Dieu, Paris, France*

Backgrounds. The discovery of the *JAK2 V617F* mutation has profoundly modified the diagnosis of myeloproliferative diseases (MPD). In essential thrombocythemia (ET), the most frequent MPD, the mutation has been found in 30 to 57% of cases studying neutrophil's DNA. *Aim*: In this study we aimed to assess the most informative cellular fraction for the *JAK2 V617F* detection. *Patients and Methods.* We explored a cohort of 260 consecutive patients referred to our institution with a suspected diagnosis of ET. We studied neutrophils and bone marrow mononuclear cells at the DNA level and platelet's RNA. The detection of the mutation consisted in a real time PCR on LightCycler followed by a melting curve analysis (sensitivity 2-4%), allowing a semi-quantitative estimation of mutated/wild type allele. Bone marrow culture assays for endogenous erythroid and megakaryocytic colony formation (EEC, EMC) were performed in 146/260 patients. Results.The mutation was found in 141/260 (54%) patients. In 82 patients both neutrophils and platelets were studied. In 32/82 patients the mutation was detected in neutrophils and 40/82 in platelets ($p=0.18$). Thus 8 patients were detected only in platelet's RNA. Using an optimised assay (sensitivity 0.8%) all of them were found mutated in neutrophils. However these patients were more easily detected in platelets. Furthermore, 16/32 (50%) had no more than 10% *JAK2* V617F allele in neutrophils. In comparison only 2/57 PV patients had the same profile. In 27 patients studied both in platelets and bone marrow, no difference was found for *JAK2* mutational status. Bone marrow cultures revealed EEC or EMC in 64/74 (86%) of *JAK2* mutated samples. EEC or EMC were also found in 15/46 (33%) *JAK2 V617F* negative samples (in platelets or bone marrow). *Conclusion*. In the context of suspected ET, *JAK2 V617F* mutation is more easily detected in platelets. The presence of EEC or EMC in *JAK2* non mutated cases supports the idea of another underlying molecular defect. Therefore bone marrow cultures and morphology remain usefull for the diagnosis of these MPDs.

0967

UPDATE OF THE GERMAN ESSENTIAL THROMBOCYTHAEMIA (ET) STUDY: INCIDENCE OF COMPLICATIONS DURING LONG-TERM FOLLOW-UP

M. Griesshammer,[1] E. Lengfelder,[2] R. Hehlmann,[2] A. Reiter,[2] H. Beneke,[1] H. Gisslinger,[3] H. Döhner,[1] H. Heimpel[1]

[1]*University of Ulm, Ulm, Germany;* [2]*University of Heidelberg, Mannheim, Germany;* [3]*University of Wien, Wien, Austria*

The German ET-Study is a prospective, randomized multicenter trial with 31 participating centers recruiting 123 patients until 1999. Patients were stratified according to a previous history of ET related complications or a platelet count > or < 1500 G/L in high or low-risk ET patients. ET patients were regarded as high-risk ET patients, if there has been a previous history of ET related complications or if the platelet count was > 1500 G/l. These patients were randomized either to interferon α (IFN) or hydroxyurea (HU). Low risk ET-patients were defined as ET patients with no ET related symptoms and a platelet count < 1500 G/l. These patients were observed until ET related complications did occur or until the platelet count increased above 1500 G/l. In total 123 patients with a newly diagnosed ET according to the PVSG criteria and no prior cytoreductive treatment were recruited. Out of these 123 patients, 55 had a high-risk ET and were randomized to either HU (n= 27) or IFN (n= 28). The remaining 68 patients had a low-risk ET. After a median follow-up of 6 years (range 1-10 years) 13 low risk ET patients developed ET-related symptoms (8 thromboembolic episodes and 5 microcirculatory disturbances). This resulted in a total complication rate of 3.5% per 100 patient-years and in a rate of 2.0% per 100 patient-years for thromboembolic complications alone. No major bleedings were observed. ET-related complications were significantly dependent on age (age > 60 years, $p=0.001$) or the presence of ≥2 cardiovascular risk factors ($p=0.006$). After a median follow-up of 6.6 years (range 0.2-11.1 years) the total complication rate in high risk patients was 3.2% per 100 patient-years for IFN and 3.5% per 100 patient-years for HU treated patients, respectively ($p=0.5$). Age > 60 years ($p=0.01$) or the presence of ≥1 cardiovascular risk factors ($p=0.026$) were associated with a significant higher risk of ET related complications in all high risk patients. After 3 years, half of the

IFN treated patients discontinued IFN due to side effects. Two patients transformed into blast crisis while on HU. There was no patient in the IFN group developing a blast crisis. In summary, HU remains the standard treatment for high risk ET patients although the potential leukemogenicity of this drug still remains a matter of concern. In low risk ET patients a watch and wait strategy is still justified. Higher age (> 60 years) and/or the presence of cardiovascular risk factors at diagnosis are significantly associated with a higher complication rate in both high and low risk ET.

0968

A PHASE II STUDY OF NILOTINIB (AMN107), A NOVEL TYROSINE KINASE INHIBITOR, ADMINISTERED TO PATIENTS WITH SYSTEMIC MASTOCYTOSIS

A. Hochhaus,[1,2] N. Gattermann,[3] O.G. Ottmann,[4] P.H. Erben,[2] M. Schatz,[2] T. Schimansky,[5] T. Rafferty,[5] L. Alland,[5] A. Gratwohl,[6] G. Follows,[7] G. Verhoef[8]

[1]*Fak.Klin.Med. Mannheim, Mannheim, Germany;* [2]*III. Med. Klinik, Mannheim, Germany;* [3]*Universität Düsseldorf, Düsseldorf, Germany;* [4]*Goethe-Universitat, Frankfurt, Germany;* [5]*Novartis, East Hanover, NJ, USA;* [6]*Universität Basel, Basel, Switzerland;* [7]*Addenbrook's Hospital, Cambridge, United Kingdom;* [8]*UZ Gasthuisberg, Leuven, Belgium*

Backgrounds. Systemic mastocytosis is a clonal disorder characterized by constitutive activation of c-Kit based on point mutations and is characterized by mast cell infiltration of extracutaneous organs. Nilotinib is a novel aminopyrimidine which potently inhibits Bcr-Abl, as well as the PDGF-R and c-Kit kinases. *Aim:* This study was designed to evaluate the safety and efficacy of nilotinib administered at an oral dose of 400 mg twice daily. *Methods.* This is a Phase II, open-label study of SM patients with specific disease criteria and with a clinical indication for treatment. Results. Preliminary data are available for the first 23 (11 f, 12 m) out of 53 patients currently enrolled in the study. The median age is 49 (range 33-78) years and the median time from diagnosis of SM was 27 (range 1 to 292) months. Of the patients with data available 17 pts had a c-kit D816V mutation in bone marrow cells or extracutaneous organs. The median exposure to nilotinib was 144 days. Treatment is ongoing for 18 (78%) patients; 5 (22%) have discontinued; 3 (13%) for adverse events and 2 (9%) withdrew consent. There were three (13%) responses reported (2 incomplete remission and 1 minor response) based on serum tryptase, bone marrow mast cell counts and improvement of clinical symptoms. Baseline mutation data are available for 2 of the 3 responding patients and revealed the c-Kit D816V mutation. Anemia was reported in 2 (9%) patients. Adverse events occurring in ≥10% of patients included headache 52% (n=12), fatigue 39% (n=9), nausea 35% (n=8), vomiting, pruritis 30% (n=7 each), muscle spasms 26% (n=6), diarrhea, upper abdominal pain, rash 22% (n=5 each), dizziness, extremity pain dyspnea, myalgia, increased ALAT 17% (n=5 each), bone pain, abdominal pain, cough, hard feces, pustular rash 3% (n=3 each) and hypotension in 3 patients (13%). Overall Grade 3/4 adverse events included headache, pruritis, hypotension, dyspnea, myalgia, increased ALAT 9% (n=2 each), fatigue, muscle spasms, diarrhea, dizziness and extremity pain 4% (n=1 each). There were no deaths. *Summary/Conclusions.* These data suggest that nilotinib has clinical activity and an acceptable safety and tolerability profile in patients with systemic mastocytosis.

0969

IMATINIB-MESYLATE THERAPY FOR SYSTEMIC MASTOCYTOSIS: RELATIONSHIP TO C-KIT MUTATIONAL STATUS

P. Musto,[1,3] M. Rondoni,[2] P. Piccaluga,[2] S. Soverini,[2] S. Paolini,[2] A. Falcone,[3] G. Sanpaolo,[3] M.L. Barone,[1] R. Guariglia,[1] L. Ciuffreda,[1] G. Pietrantuono,[1] P. Vivaldi,[4] F. Lauria,[5] M. Baccarani,[2] G. Martinelli[2]

[1]*CROB, Rionero, Italy;* [2]*'L and A Seragnoli' Hematology Institute, Bologna, Italy;* [3]*IRCCS 'Casa Sollievo della Sofferenza, S. Giovanni Rotondo (FG), Italy;* [4]*Istituti Ospedalieri, Trento, Italy;* [5]*Chair of Hematology, Seina, Italy*

Background. Systemic mastocytosis (SM) includes an heterogeneous group of neoplastic disorders characterized by an abnormal mast cell accumulation in various tissues and by both indolent or aggressive clinical outcome. SM has been supposed to be associated with two classes of constitutive activating c-kit somatic mutations: the so-called *enzymatic site* type (EST) mutations, affecting the structure of the catalytic portion of the kinase (e.g., D816V), and the *regulatory* type (RT) mutations, affecting the regulation of an otherwise normal catalytic site (e.g., V560G). *Aims*. Since c-kit is a transmembrane receptor-type tyrosin kinase, we aimed to test the hypothesis of whether an inhibitor blocking constitutive c-kit activation, such as imatinib, could have therapeutic activity in SM and whether c-kit mutational status could have importance for response. *Methods.* We report on nine patients treated with imatinib who met the major classification criteria for SM, who were symptomatic and who had a biopsy-proven evidence of disease. Six of them were male and three female, age ranged from 33 to 76. Organ involvement included skin, bone, stomach, bowel, bone marrow, spleen, lymphnodes, lung and hearth, variously combined in different patients. Two patients had elevated eosinophils in their peripheral blood (45% and 19%, respectively). All patients resulted to be negative for the FIP1L1-PDGFRα fusion transcript, which characterizes hypereosinophilic syndromes (HES) with high sensitiveness to imatinib. The drug was given at the dose of 400 mg/die for a median period of 3 months (range 1,5-5 months). *Results.* Before therapy, mastocyte cells from six patients were found positive for D816V mutant of c-kit. None of these patients achieved significant clinical benefits from imatinib therapy. Regarding the three subjects without D816V c-kit mutations, one patient, with elevated count of eosinophils in peripheral blood, showed an initial response to imatinib, but lost it after one month from the beginning of treatment. A second patient had a significant reduction of splenomegaly, but rapidly relapsed and died of progressive disease. The third patient without D816V mutation showed a prolonged significant reduction of skin lesions and marrow mastocytosis. *Conclusions.* Our observations suggest that imatinib treatment is ineffective for SM characterized by EST c-kit mutation, even if associated with hypereosinophilia, while patients without such a mutation may have some clinical benefit. A complete evaluation of the molecular pattern in SM treated with imatinib, including RT mutations (e.g., V560G and F522C), is currently in progress.

0970

SDF1-3A POLYMORPHISM IN MYELOFIBROSIS WITH MYELOID METAPLASIA: GENOTYPE-PHENOTYPE CORRELATION

M. Marchetti,[1] M.L. Biondi,[2] M. Massa[1], R. Campanelli,[1] G. Bergamaschi,[1] E. Bonetti,[1] V. Rosti,[1] A. Pecci[1], G. Gerli,[2] G. Barosi,[3] R.I.M.M., on behalf of the Italian Registry of Pavia, Italy

[1]*IRCCS Policlinico San Matteo, Pavia, Italy;* [2]*Ospedale San Paolo, Milan, Italy*

Background : The pathogenesis of myelofibrosis with myeloid metaplasia (MMM) is still unknown. Stromal-derived factor-1 (SDF1) is a chemokine constitutively produced by bone marrow stromal cells and playing a key role in hematopoiesis. Position 801 (3' untranslated region) of the *SDF1* gene commonly contains a G to A transition (SDF1-3'A). Such a polymorphism might be implicated in *SDF1* production or in its activity. *Methods.* Seventy-one consecutive patients referring to the Italian Registry of MMM from October 2005 to February 2006 were sampled 10 mL peripheral blood. SDF1 and JAK2 genotypes were determined with PCR-RFLP assays. Plasma SDF1α levels were determined with commercial ELISA kit. By cytofluorimetric analysis we evaluated the portion of circulating $CD34^+$ cells expressing the *CXCR4* antigen on the membrane ($CD34^+$ $CXCR4^+$) and the portion expressing it intracellularly ($CD34^+$intra$CXCR4^+$). *Results.* The patients had a median age of 54 years and were sampled at a median of 24 months after diagnosis. Fifteen patients had a previous ET and five a previous PV (secondary MMM). Overall, 25 and 5 patients showed SDF1-3' AG and AA genotypes, respectively: the SDF1-3'A allelic frequency was 25%, not significantly different from the general population (*Gerli et al., 2005*). The *SDF1-3'A* allelic frequency was 75% in patients with secondary MMM, significantly higher than the general population ($p<0.0001$) and primitive MMM ($p=0.003$), and even higher than the ET or PV patients reported by Gerli *et al*. ($p=0.0002$). This frequency was 8% in patients with chromosomal abnormalities versus 26% in those with normal chromosomes ($p=0.19$). Patients carrying the SDF1-3'A polymorphism showed smaller spleen size (16 vs 21cm longitudinal diameters; $p=0.02$) and lower leukocyte counts at diagnosis. No significant correlation was found between SDF1-3' genotype and SDF1α plasma levels, while the number of A alleles inversely correlated with the percentage of $CD34^+$intra$CXCR4^+$ cells, particularly in patients with secondary MMM ($p=0.004$), who also showed higher percentages than patients with idiopathic MMM ($p=0.009$). The number of A alleles also correlated, positively, with the percentage of $CD34^+$ $CXCR4^+$ cells, but only in patients with idiopathic MMM ($p=0.02$). Twenty-one and six patients were *JAK2-V617F* heterozygotes and homozygotes, respectively. All the 5 SDF1-3' AA patients also carried a heterozygote mutation of *JAK2*, however, the SDF1-3'A allelic frequency was independent of *JAK2* mutation. Among the JAK2 wt/wt patients, the SDF1-3' GG genotype was

detected in 14, only 1 of whom had a secondary MMM (p=0.02): such patients showed higher values of leukocyte count (p=0.08), CD34 count (p=0.03), serum LDH (p=0.02) and spleen size, a higher frequency of severe anemia (hemoglobin< 9g/dL; p=0.02), higher SDF1α plasma levels (p=0.04), lower percentage of CD34$^+$ *CXCR4*$^+$ cells, higher percentage of CD34$^+$ intraCXCR4 cells and a trend to a lower bone marrow cellularity, as compared with SDF1-3' AG or AA patients. Similar phenotypic differences between the GG and the AG/AA genotypes were detected both in idiopathic and secondary MMM patients, and also in the patients with mutated JAK2. *Conclusions.* The *SDF1-3'A* polymorphism is highly frequent in secondary MMM and influences MMM phenotype, favoring a less intense myeloproliferation and less severe anemia.

0971

TOPOGRAPHY OF INTRAMEDULLARY HEMATOPOIESIS IN MYELOFIBROSIS WITH MYELOID METAPLASIA: RELEVANCE OF 99TC-BW250/183 IMMUNOSCINTIGRAPHY

M. Marchetti,[1] C. Aprile,[2] B. Gerco,[2] G. Barosi,[1] On behalf of the R.I.M.M.[3]

[1]*IRCCS Policlinico San Matteo, Pavia, Italy;* [2]*Fondazione S.Maugeri, Pavia, Italy;* [3]*Italian Registry of Myelofibrosis With Myeloid Metaplasia, PAVIA, Italy*

Backgrounds. Myelofibrosis with myeloid metaplasia (MMM) is a rare chronic myeloproliferative disease characterized by both myeloproliferative and myelodepletive features: myeloproliferation typically includes enhanced spontaneous mobilization of hematopoietic progenitor cells (HPC) from the bone marrow (BM) and their homing into extramedullary sites (mainly spleen); myelodepletion results from exhaustion of both BM and extramedullary hemopoiesis. *Aims.* To investigate the extent and distribution of hemopoiesis in MMM patients and to capture its relationship with BM fibrosis, HPC mobilization and clinical severity. *Methods*. Immunoscintigraphy employing a dual-head camera was performed 120-260 minutes (median 180 minutes) after administration of 553-830 MBq (median 700 MBq) 99mTc-BW250/183, corresponding to 0.3-0.5 mg. Hemopoietic function in the central compartment (sacrum) was described by *subtypes* (normal, increased or decreased) and by the sacrum-to-soft tissue uptake ratio (UR) (*Huic et al., J Nucl Med 1997)*. The degree of peripheral BM displacement (limbs) was described through 5 *types* (I to V) (*Huic et al., J Nucl Med 1997*). Chi-square and *ANOVA* tests were adopted for descriptive statistics. *Results*. Twenty-three MMM patients (10 females, median age 55 years) were studied. Eleven patients showed a reduced uptake by the central BM compartment: they had a higher WHO fibrosis grade (p=0.008), lower hemoglobin values (p=0.012) and lower platelet counts (p=0.007) than patients with a preserved central compartment. Patients with an exhausted central compartment at immunoscintigraphy also showed a significantly higher mobilization of HPC into peripheral blood: CD34$^+$ count was 0.86% in patients with a depressed central compartment versus 0.12% in those with a preserved one (p=0.029). Accordingly, immature myeloid cells or blasts (9.5% versus 0.3%; p=0.005), spleen size (9.3 vs 2.4 centimeters from costal arc; p=0.007) and LDH values (1168 vs 566 UI/l; p=0.011) were significantly higher. Among the patients with a depressed central compartment, those who lost also peripheral BM function (type V) showed a more severe myelodepletion and more intense HPC mobilization. On the opposite side, among the 12 patients with a preserved central compartment, the 8 ones with a mild peripheral marrow displacement (type I-II) showed absent or mild fibrosis (WHO 0-1), elevated platelet counts, normal to high hemoglobin values, minimally enlarged spleens and no hints of increased HPC mobilization (CD34$^+$ <0.1%; no blasts; ≤1% immature myeloid cells). From these data it appears that progressive fibrosis and exhaustion of central BM is accompanied by a gradual displacement of hemopoiesis into the peripheral bone compartment and spleen and by derangement of HPC trafficking. *Conclusions.* BM immunoscintigraphy tracks MMM clinical features and may help staging patients, understanding the biology of the disease and targeting therapies

0972

THE REGISTRO ITALIANO TROMBOCITEMIE: PRELIMINARY ANALYSIS OF THE FIRST 650 ENROLLED PATIENTS

L. Gugliotta,[1] A. Tieghi,[1] S. Franceschetti,[1] C. Santoro,[1] P. Carluccio,[1] R. Ciancia,[1] E. Rumi,[1] F. Palmieri,[1] E. Antonioli,[1] D. Cilloni,[1] F. Radaelli,[1] N. Vianelli,[1] M.R. Villa,[2] A.B. Federici,[3] M.L. Randi,[4] A. Candoni[1]

[1]*Hematology, Reggio Emilia, Italy;* [2]*Hematology SG, Naples, Italy;* [3]*Hemophilian and Thrombosis, Milan, Italy;* [4]*Internal Medicine, Padua, Italy*

Background. Many epidemiological, diagnostic, prognostic and therapeutical data were obtained by the Italian Registry with the retrospective study performed in over 2000 Essential Thrombocythemia (ET) patients, mainly diagnosed according to the PVSG criteria and treated with potentially leukemogenic drugs. Now are claimed updated data in ET patients diagnosed according to the WHO criteria and treated more largerly with non leukemogenic molecules. *Aims*. The RIT belonging to the GIMEMA Group has been activated in order to registry italian ET patients; to improve the diagnosis appropriateness (WHO criteria) by performing a centralized revision of the bone marrow biopsies; to promote the acquisition of biological data; to evaluate the compliance to the therapeutical guidelines of SIE, SIES, GITMO; to monitor particularly the ET patients receiving Interferons α and Anagrelide; to evaluate cases of pregnancy, pediatric age and familiarity; to identify new prognostic factors (JAK2 mutation, clonality, etc); to create a network for activation of new clinical and biological studies. *Methods*. The RIT, co-ordinated by the Hematology Unit of Reggio Emilia, is a web-based registry that besides a public area comprehends a database of italian ET patients. The data, with respect of the privacy rules, are object of validation and analisys by various RIT Expert Subcommittees. *Results.* Eighty Hematological Centers adhered to the RIT and 650 patients have been registered since June 2005. In the first 505 analysed cases the ET diagnosis was done according to the PVSG (92%) and WHO (8%) criteria.The patients, 311 females and 194 males, had age <40 yr (17%), 40-60 yr (34%), 60-70 yr (20%), >70 yr (29%) with median age 59 years. At diagnosis the platelet count was >1000×10^9/L in 27% of cases (mean 915). Few patients had prior thrombosis (4.4%; major 2.4%) and prior hemorrage (1%). The rate of high risk patients, on considering age >60 yr and/or previous thrombosis and/or PLT count >1500×10^9/L, was 56% (64% on considering the PLT count cut-off of 1000×10^9/L). The patients shown gereral thrombotic risk factors (69%), disease related symptoms (42%) and splenomegaly (27%). Data on the bone marrow biopsy permitted to identify as true ET (WHO criteria) the 27% of the cases. The cytogenetic study documented a normal karyotype in all the 274 evaluated cases. The bcr-abl transcript was absent in all cases. Fifty-nine pregnancies have been reported. Aspirin was administered in 70% of cases and cytoreduction was performed in 63% of cases: Hydroxyurea 61%, Anagrelide 12%, Interferons α 11%, Pipobroman 4%, Busulfan 2%. The follow-up is too short to analyse data. *Conclusions*. Many (80) of the Italian Hematological Centers have been accredited by the RIT. In 92% of ET patients diagnosis was still done according to the PVSG criteria, but the ongoing revision of the bone marrow biopsies will permit a reclassification according to the WHO criteria. Improvement of the diagnostic approach is expected since the harvest of biological material as been activated. A separate analisys is ongoing for specific series of patients treated with anagrelide and interferons α.

0973

FAMILIAL CHRONIC MYELOPROLIFERATIVE DISORDERS: CLINICAL PHENOTYPE AND JAK2 (V617F) MUTATION STATUS

F. Passamonti, E. Rumi, D. Pietra, S. Boggi, C. Elena, L. Arcaini, M. Della Porta, M. Bonfichi, E. Boveri, C. Pascutto, M. Lazzarino, M. Cazzola

IRCCS Policlinico S. Matteo Pavia, Pavia, Italy

Background. Philadelphia (Ph)-negative chronic myeloproliferative disorders (CMD) include polycythemia vera (PV), essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF). CMD are acquired diseases due to a somatic stem cell mutation leading to clonal expansion of myeloid precursors. A gain-of-function mutation of the Janus kinase 2 (JAK2) gene has been recently recognized as a pathogenetic event of CMD. Besides sporadic cases, one or several CMD may affect different relatives of the same family, namely familial CMD. The probability that two CMD occur in the same family as independent events is really low (estimated annual incidence: $10^{-5}\times10^{-5} = 10^{-10}$). This suggests the presence of genetic predisposition for somatic mutations leading to CMD-like syndromes in families. Although familial cases carrying JAK2 (V617F) mutation have been reported, the frequency of this mutation in CMD families and its role in disease-causing remain to be defined. *Aims*. The aim of this study was to evaluate the clinical features and outcome of familial chronic myeloproliferative disorders, to assess the frequency of JAK2 (V617F) within familial cases, and to define its role in disease-causing. Patients and Methods. Sixteen pedigrees were evaluated for clinical and molecular studies. Pedigrees included 11 families with an homogeneous phenotype (polycythemia vera in 8; essential thrombocythemia in 3), and 5 with a mixed CMD phenotype. Collecting DNA from granulocytes and T lymphocytes, we detected JAK2 (V617F) by use of quantitative mutation-specific polymerase chain reaction and X-chromosomal clonality markers HUMARA, PGK, and IDS. *Results*. Clinical features at diagnosis and outcome did not differ between familial and sporadic CMD. JAK2 (V617F) ranged from 5.3 to 91.5%: the higher value being detected in post-polycythemia myelofibrosis. Distribution of mutant JAK2 within the same pedigree displayed an homogeneous pattern (5 families), or a discordant one (4 families). T cells DNA did not carry mutant alleles. All clonal CMD females, except one with ET, were *JAK2 (V617F)*-positive. One polyclonal ET was JAK2 (V617F)-positive (low gene dosage: 5.3%). One PV patient was polyclonal and *JAK2 (V617F)*-negative. Screening of healthy relatives identified 2 subjects with early-polycythemia. *Conclusions.* These data show that patients with familial CMD have clinical features and outcome overlapping with those of sporadic cases. An extended study of the pedigrees of CMD patients is warranted to ascertain the real frequency of familial cases. *JAK 2 (V617F)* is a somatic mutation that at least in a portion of familial patients with ET or CIMF does not appear the disease-initiating event.

0974

SPANISH REGISTRY OF ESSENTIAL THROMBOCYTHEMIA

M. Giralt, C. Besses, C. Burgaleta, F. Carbonell, L. Hernández-Nieto, G. Ramírez, On behalf of GEMFIN

Gemfin, Zaragoza, Spain

Background. The RETE (Registro Español de Trombocitemia Esencial) is an open, retrospective and multicenter registry of patients with essential thrombocythemia (ET) treated with Anagrelide, designed and promoted by GEMFIN (Grupo de Estudio de Enfermedades Mieloproliferativas Filadelfia Negativas). *Aim*. The aim of the registry was to assess retrospectively the efficacy and safety of Anagrelide in newly diagnosed ET patients, as well as in those intolerant/refractory to their current therapy. *Patients and Methods*. 411 ET patients from 54 Centers were included. ET was diagnosed according to PVSG criteria (1997). Risk groups were defined as follows: high risk (patients with an age above 60 years and/or history of thrombosis), low risk (patients with an age below 60 years, no history of thrombosis and platelet count < 1.5×10^9/L) and intermediate risk (patients that belonged neither to high nor low risk groups). Response to treatment was defined as complete remission (CR) when a platelet count equal or less than 400 x 109/L was achieved; partial remission (PR) when the platelet count was between 400 and 600×10^9/L and no response (NR) when platelet count was > 600 x 109/L. All these data and adverse events were collected along 2004 and sent for blind analysis by two external data manager. *Results.* The median age at diagnosis was 50 years (14-92) and 253 patients were females (61.6%). Patients were stratified at diagnosis in the following risk groups: 167 (40.6%) high-risk, 97 (23.6%) intermediate-risk and 147 (35.8%) low-risk. At presentation, 114/411 (27.7%) manifested thromboembolic complications and 73/411 (17.7%) showed bleeding events. Evolution to myelofibrosis, polycythemia vera and acute leukemia/MDS was observed in 20, 4 and 9 patients, respectively. The reason/s to initiate Anagrelide were thrombocytosis (n=282; 68.6%), age (n=238; 57.9%), bleeding (n=40; 9.7%), thrombosis (n=80; 19.4%) and resistance to previous therapy in 158 patients (38.4%). Median starting, maintenance and maximum Anagrelide dosage was 1.5 mg, 1.5 mg and 2.0 mg, respectively. In the whole group, CR was obtained in 219 patients (53.3%) and PR in 113 (27.5%), giving an overall response (OR) rate of 80.8%. Median time (months) to reach CR was 6.3 months and 5.5 months for PR. The rate of responses (CR;PR;OR;NR) in Anagrelide naïve patients (n=110) was 56.4%; 30.0%, 86.4% and 13.6% while the rate of responses in previously treated patients was 52.2%, 26.6%, 78.7% and 20.6%. The incidence of adverse events appearing in more than 10% of patients was: headache (61%), tachycardia (26%), palpitations (32%), oedema (17%), anemia (17%), diarrhea (14%), vertigo (13%) and dyspepsia (11%). Discontinuation of Anagrelide was reported in 36.2% of patients and was due to headache (13%), tachycardia (10%), palpitations (9%), oedema (8%) and anemia (3%). The incidence of thrombosis and hemorrhage observed during Anagrelide treatment was 5.6% and 6.8%, respectively. *Summary*. RETE study showed: a) a high hematological response rate in Anagrelide naïve and previously treated patients, (86.4% and 78.7%, respectively) and, b) an incidence and type of adverse events in agreement with those reported.

Funding. Shire Ibérica.

SIMULTANEOUS SESSIONS

Acute myeloid leukemia and myelodysplastic syndromes - Clinical

0975

CYTOGENETICS AND AGE ARE THE MAIN DETERMINANTS OF OUTCOME IN INTENSIVELY TREATED ACUTE MYELOID LEUKEMIA PATIENTS OLDER THAN 60 YEARS: RESULTS FROM AMLSG TRIAL AML HD98-B

R.F. Schlenk, S. Kayser, M. Morhardt, K. Döhner, H. Döhner, S. Fröhling

University of Ulm, Ulm, Germany

Backgrounds. Karyotype at diagnosis provides the most important prognostic information in younger adults with acute myeloid leukemia (AML). However, there are few data available looking in particular at patients (pts.) above 60 years of age. *Aims.* Evaluation of the prognostic value of cytogenetics and additional variables in elderly AML patients. *Methods.* We prospectively analyzed 361 elderly pts. with newly diagnosed AML. Chromosome banding was performed using standard techniques. To improve cytogenetic diagnostics, all specimens were also analyzed by FISH using a comprehensive DNA probe set for the detection of the most relevant AML-associated genomic aberrations: inv(3)/t(3;3), t(8;21), t(9;22), t(11q23), t(15;17), inv(16)/t(16;16), +3q, +4q, del(5q), del(7q), +8q, +11q, abn(12p), del(13q)/+13q, del(17p), del(20q), +21q, +22q, del(Xq). All pts. were treated within the AMLHD98B treatment trial and received intensive induction and consolidation therapy. Pts. exhibiting a t(15;17) received an age-adjusted AIDA-regimen. Median follow-up time was 57 months. The median age was 67 years (range 60-85 years). *Results.* 161 pts. had a normal karyotype (45%); 48 pts. (13%) exhibited the balanced translocations t(8;21) (n=12), inv(16) (n=14), t(15;17) (n=11), or t(11q23) (n=11); in the absence of these balanced translocation, 73 pts. exhibited a single aberration, 179 pts. two aberrations, and 61 pts. a complex karyotype (≥3 aberrations; including 44 pts. with 5 or more aberrations). Analyses were normalized to the complete remission (CR) rate (52%), cumulative incidence of relapse (CIR) (77%) after 2 years and overall survival (OS) (19%) after 3 years of pts. with normal karyotype. Pts. exhibiting a t(15;17) showed a significantly better CIR (29%) and OS (55%), whereas pts. with the other balanced translocations [t(8;21), inv(16)/t(16;16) and t(11q23)] did not differ from pts. with normal karyotype. The limited backward selected Cox-model for OS [t(15;17) excluded] revealed two risk groups: standard-risk [normal karyotype, t(8;21), inv(16), t(11q23), +8 and +11 in absence of a complex karyoytpe] and high-risk [all other aberrations]. The second main determinant for prognosis was age with a cut point at 70 years defined by maximally selected log-rank statistics (p<0.001). Stratification of the patients according to cytogenetic risk group and age as dichotomized variable resulted in 5 prognostic groups: i) APL CR 73%, OS 55%, ii) <70 yrs./standard risk CR 62%, OS 24%, iii) <70 yrs./high risk CR 21%, OS 6%, iv) >70 yrs./standard risk CR 39%, OS 3%,v) >70 yrs./high risk CR 15%, OS 2%. *Conclusion*: Our risk classification system based on cytogenetics and age identified a large proportion of elderly patients with AML who did not benefit from intensive chemotherapy.

0976

5-AZACITIDINE INDUCES REMISSIONS IN PATIENTS WITH TRANSFUSION DEPENDENT MYELOPROLIFERATIVE DISEASES AND IN PATIENTS WITH ACUTE MYELOID LEUKEMIA REFRACTORY TO OR NOT ELIGIBLE FOR INTENSIVE CHEMOTHERAPY

H.K. Al-Ali, S. Schwind, C. Becker, C. Christl, D. Niederwieser

University of Leipzig, Leipzig, Germany

Backgrounds. Epigenetic modulation of gene function is a powerful cellular mechanism. An association between methylation of the p15 ink4b gene promotor and risk for acute myeloid leukemia (AML) transformation in myelodysplastic syndrome (MDS) has been suggested. The DNA hypomethylating pyrimidine analogue 5-azacitidine may reduce hypermethylation and induce re-expression of key tumor suppressor genes in MDS. Azacitidine induces remarkable responses in 60% of patients with MDS. However, little is known about the clinical activity of azacitidine in patients with AML refractory to or not eligible for intensive chemotherapy as well as in patients with myeloproliferative diseases (MPD). The safety and efficacy of azacitidine in this cohort of patients are being assessed at the University of Leipzig. Patients and Methods. 19 patients (13 m/6 f), median age 71 years (range 58-78 years) received 75 mg/m^2 azacitidine subcutaneously for 5 days every 4 weeks mostly in an outpatient setting at the University of Leipzig. Diagnoses were AML, n=15 (79%) [not eligible for chemotherapy, n=8, refractory disease, n=5, partial remission only, n=2], MPD, n=4 (21%) [transfusion dependent, n=3, MPD with thrombocytopenia, n=1]. In patients with AML, 11 (73%) suffered from secondary AML. Abnormal cytogenetics were present in 7 (37%) patients [complex aberrations, n=4, trisomy 8, n=2, iso(17)(q10), n=1]. Results. Up till now, a total of 55 treatment cycles were applied. Azacitidine was well tolerated with a few non-hematologic side effects (constipation grade I, n=3, skin irritation at the site of injection grade I, n=2, parasthesia grade I, n=1, transient elevation of SGPT/SGOT grade I, n=4, transient creatinin elevation grade I, n=2). Only one reversible grade IV liver toxicity was observed. Transient treatment related thrombocytopenia and pancytopenia occurred in 5 (26%) and 2 (11%) patients respectively. After a median number of 3 treatment cycles/patient, clinical efficacy of azacitidine could be evaluated in 17 patients. In patients with AML, remissions were achieved in 6/13 (46%) (CR, n=4, PR, n=2). Stable disease was achieved in 2 patients. Five (38%) patients were refractory to treatment. For patients with MPD, PR was achieved in 3 patients and stable disease in one. For the entire cohort, 55% of transfusion dependent anemias and thrombocytopenias resolved under therapy. Interestingly, complete remissions in patients with AML were achieved after 1-2 treatment cycles. Patients refractory to conventional chemotherapy tended to do worse than patients who received azacitidine as first line treatment. *Conclusion*: Azacitidine applied in an outpatient setting to patients with AML and MPD with a dismal prognosis was well tolerated and could induce complete and partial remissions. It may be a promising new treatment modality for patients with AML and MPD. A larger number of patients and longer follow up are needed to confirm these data, define the number of treatment cycles required and clarify whether a leukaemia-free survival correlates with an improved overall survival in this group of patients.

0977

PHASE IB STUDY OF PKC412, AN ORAL FLT3 KINASE INHIBITOR, IN SEQUENTIAL AND SIMULTANEOUS COMBINATIONS WITH DAUNORUBICIN AND CYTARABINE INDUCTION AND HIGH-DOSE CYTARABINE CONSOLIDATION IN NEWLY DIAGNOSED PATIENTS WITH AML

T. Fischer,[1] F. Giles,[2] R. Paquette,[3] G. Schiller,[3] G. Ehninger,[4] C. Schiffer,[5] J. Cortes,[2] H. Kantarjian,[2] D. DeAngelo,[6] F. Heidel[1], P.S. Cohen,[7] R. Yu,[7] S. Bilic,[7] L. Zhang,[7] P.S. Phillips,[7] R.M. Stone[6]

[1]*University Hospital Mainz, Mainz, Germany;* [2]*MD Anderson Cancer Center, Houston, TX, USA;* [3]*UCLA Medical Center, Los Angeles, CA, USA;* [4]*Universitaetsklinikum Carl Gustav Carus, DRESDEN, Germany;* [5]*Karmanos Cancer Institute, DETROIT, MI, USA;* [6]*Dana Farber Cancer Center, BOSTON, MA, USA;* [7]*Novartis Oncology, EAST HANOVER, NJ, USA*

Backgrounds. Activating mutations in FLT3 (fms-like tyrosine kinase), either an internal tandem duplication (ITD) in the juxtamembrane region or a point mutation in the activation loop, occur in leukemic blasts from 25-35% of AML patients, are associated with poor prognosis, and represent an attractive therapeutic target. PKC412 is a multi-targeted kinase inhibitor which has clinical activity in mutant (reduction in peripheral blasts in 70%) and wild type (reduction in peripheral blast in 30%) AML, but rarely produces remissions (Stone et al., Blood 2005). *Aims and Methods.* We combined DA induction (daunorubicin 60 mg/m^2 d 1-3 and cytarabine 100 mg/m^2/d by IVCI × 7d) and post-remission HD-ARAC (cytarabine 3 gm/m^2/3h q 12h, d1,3,5 for 3 cycles) plus PKC412 in newly diagnosed FLT3 mutated (FLT3mut) and FLT3wild type (FLT3WT) AML patients < 60 years old in a Phase Ib trial to investigate toxicity and efficacy. Results of earlier experience using PKC412 100 mg po bid were reported previously (*Giles et al., ASH 2004*). This is an updated report of 23 patients treated with PKC412 at a reduced dose of 50 mg po bid given on day 8-21 (arm 1) or day 1-7, 15-21 (arm 2), plus DA and HD-ARAC. Eight out of 23 patients (35%) were FLT3mut , and 15/23 (65%) were FLT3WT as determined by D-HPLC. Results. None of the 19 patients evaluable for safety had drug-related death; the most common drug related grade 3/4 non-hematologic toxicities were transient elevations of glucose (16%), AST (16%), bilirubin (11%), ALT (11%), and decreases in potassium (21%), phosphate (11%) and calcium (11%); no grade 3 or 4 nausea, vomiting or pleural effusion were recorded. Twenty-three patients were evaluable for response: 9/12 (75%) achieved CR in Arm 1 and 9/11 (82%) achieved CR in Arm 2. Seven out of 8 (88%) FLT3mut patients and 11/15 (73%) FLT3WT patients achieved CR, trending towards a higher CR rate for the PKC412/induction chemotherapy

regimen administered to FLT3mut patients in comparison to FLT3WT patients. Accrual to this trial was recently completed (N=40) and updated information on safety and CR rates will be presented. Conclusions. PKC412 at 50 mg po bid given either sequentially or simultaneously in combination with DA and HD-ARAC can be given safely with good tolerability in newly diagnosed patients with FLT3mut and FLT3WT AML < 60 years old. This combination merits further study in a randomized fashion (± PKC412) particularly in patients with FLT3mut AML.

0978

ARSENIC TRIOXIDE (ATO) IS SAFE AND EFFECTIVE IN COMBINATION WITH LOW-DOSE ARA-C (LDAC) FOR THE TREATMENT OF ADVANCED MYELODYSPLASTIC SYNDROME (MDS) AND POOR-PROGNOSIS ACUTE MYELOID LEUKEMIA (AML) IN ELDERLY PATIENTS

G.J.R. Roboz, E. Ritchie, S. Allen-Bard, T. Curcio, J. Provenzano, M. Samuel, M. Schuster, A. Bloch, E.J.F. Feldman

Weill Medical College of Cornell Univer, New York, NY, USA

Background/Aims. Treatment outcomes for advanced MDS and elderly AML patients are generally poor. LDAC in elderly AML patients results in a CR rate of approximately 20% and possibly improved morbidity and mortality compared to conventional chemotherapy or supportive care. In MDS patients, the CR rates with LDAC are lower (10-20%) with short duration and no clear benefit over supportive care. On the basis of preclinical data suggesting a possible anti-angiogenic effect of ATO, as well as clinical data showing activity of ATO in MDS, a phase I/II study of ATO in combination with LDAC was initiated in IPSS Int-2/high risk MDS and newly-diagnosed, poor-prognosis AML patients. *Methods*. ATO was given at a dose of 0.25 mg/kg for days 1-5 and 8-12. LDAC was dose-escalated from 5 mg/m^2 SC BID to the target phase II dose of 10 mg/m^2 SC BID for days 1-14 (one treatment cycle). Patients who achieved CR after one treatment cycle were given a second, identical cycle, followed by maintenance treatment of 5 days of LDAC and 2 days of ATO every 28 days. Patients who did not achieve CR after one cycle were given a second cycle beginning between days 21-28, with the addition of ascorbic acid 1g IV within 30 minutes of the ATO infusion. *Results*. Eighty-three patients have been enrolled to date, 52 with AML and 31 with MDS. A total of 75 patients (49 AML, 26 MDS) are evaluable for response, 69 (46 AML, 23 MDS) of whom were treated with the target dose of LDAC. There were no responses in the 6 patients treated with less than the target dose. Clinical characteristics of the 46 evaluable AML patients treated at the target dose include: mean age 73 yrs (range 55-85 yrs; one patient < 60 yrs with AML and multiple medical comorbidities was included); abnormal cytogenetics 29 (66%); antecedent hematologic disorder 29 (63%); secondary disease 7 (15%). CR was achieved in 17 patients (37%) and CRp in 1 patient, for an overall response rate of 39%, with follow-up 1-11+ mos. Eight patients (44%) required 2 treatment cycles to achieve CR/CRp. Of the 46 AML patients, 5 died prior to day 30 (induction mortality = 11%), 3 (7%) of progressive disease and 2 (4%) of neutropenic sepsis. Clinical characteristics of the 23 evaluable MDS patients include: mean age 70 yrs (range 56-84 yrs); abnormal cytogenetics 17 (81%); prior therapy with 5-azacytidine 3 (13%). CR was achieved in 6 (26%) patients, follow-up 3-9+ mos. Three patients (13%) required 2 treatment cycles to achieve CR; there was 1 induction death (4%). *Summary*. The regimen was generally well-tolerated, with minimal grade 3/4 non-hematologic toxicity and no significant nausea, emesis, diarrhea or mucositis. Alopecia was not seen. Grade 4 hematologic toxicity was observed in all patients. Fluid retention occurred in 56/69 (81%) of patients. There were no clinically significant drug-related arrhythmias. The CR rate in AML was comparable to conventional chemotherapy, with improved tolerability and induction mortality; further investigation is warranted.

0979

CYTOGENETIC RESPONSES TO THE HYPOMETHYLATING AGENT, DECITABINE (DAC), IN A PHASE III TRIAL OF DAC VS SUPPORTIVE CARE (SC) IN PATIENTS (PTS) WITH MYELODYSPLASTIC SYNDROMES (MDS)

M. Kantarjian,[1] J. Issa,[1] I. Saba[2]

[1]*MD Anderson Cancer Center, HOUSTON, USA;* [2]*H. Lee Moffitt Cancer Center & Research, TAMPA, USA*

Backgrounds. Clonal cytogenetic abnormalities are detected in 40%'70% of cases of *de novo* MDS and 95% of cases of therapy-related MDS, and the incidence increases with poor risk. DAC (DacogenTM) is a cytosine analog that reverses aberrant DNA methylation, leading to re-expression of silenced tumor suppressor genes. Aims. In this analysis, we asked whether the hypomethylating agent DAC leads to cytogenetic response in MDS. Methods. We report cytogenetic response data from a Phase III randomized, open-label trial of DAC vs SC in 170 MDS pts. Eligibility requirements included confirmed MDS (de novo or secondary) fitting any of the recognized French-American-British classifications and an International Prognostic Scoring System (IPSS) score of 0.5 or more as determined by complete blood count, cytogenetics, and bone marrow assessment. Cytogenetics was assessed as a secondary endpoint, whereas primary endpoints were response rate (CR+PR) and time to AML or death. For pts with clonal abnormalities at baseline, follow-up cytogenetic evaluations at study end were available for 26 pts in the DAC arm and 21 pts in the SC alone arm. Results. As previously reported, overall response rate according to International Working Group MDS criteria was 17% (15/89) for DAC vs 0% for SC (p<0.001). Responses occurred in all IPSS groups and were also seen in pts with 5q and 7 deletions. Response rate was 13% (2/16) in pts with 5q deletions and 21% (4/19) in pts with 7 deletions. In pts without 5q or 7 deletions, response rates were 16% (11/67) and 14% (9/64), respectively. Complete cytogenetic responses were observed in 35% (9/26) of DAC pts vs 10% (2/21) of SC pts (p=0.08, Fisher's exact). Also, 1 pt receiving DAC had a minor cytogenetic response. 10/10 DAC pts with cytogenetic response had clinical benefit (6 CR, 2 PR, 1 hematologic improvement, and 1 with normalization of marrow blast count). The primary toxicity was myelosuppression. *Conclusion*: DAC induces a substantial rate of cytogenetic responses in pts with MDS, suggesting that the clinical improvements induced by this agent are related to elimination of the neoplastic clone rather than to pure differentiation effects.

Acute lymphoblastic leukemia

0980

SINGLE NUCLEOTIDE POLYMORPHISMS OF THE MTHFR (C677T), MTRR (A66G) AND VITAMIN D RECEPTOR (CDX-2/GATA) GENES ARE IMPORTANT DETERMINANTS OF OSTEOPOROSIS IN PEDIATRIC ALL PATIENTS

R.D. van Beek,[1] M.M. van der Heuvel,[1] R. de Jonge,[2]
A.G. Uitterlinden,[2] R. Pieters,[1] S.M.P.F. de Muinck Keizer-Schrama[1]

[1]*Erasmus MC-Sophia, Rotterdam, Netherlands;* [2]*Erasmus MC, Rotterdam, Netherlands*

Background. Corticosteroids and methotrexate have adverse effects on growth and bone mineralisation. Aim and methods. The influence of single nucleotide polymorphism's (SNP's) in the vitamin D receptor gene (VDR; 5' Cdx-2/GATA and 3' BsmI, ApaI, TaqI), methylenetetetrahydrofolate reductase gene (MTHFR; C677T and A1298C), methionine synthase reductase gene (MTRR; A66G), estrogen receptor gene (ER; PvuII/XbaI), glucocorticoid receptor gene (GR; BclI) and collagen type I gene (COLIA1; Sp1 binding site), on bone mineral (apparent) density (BM(A)D) and fracture rate was evaluated in 68 children (38 boys, 30 girls; mean age 7.4 yr) with ALL, treated with a 2-year dexamethasone-based protocol without cranial irradiation. BMD of lumbar spine (LS) and total body (TB) were measured using DXA-scan four times during therapy and one year after therapy and expressed as standard deviation scores (SDS). Results. ER, COLIA1 and GR did not influence BMD. Carriers of the MTHFR 677 T-allele had a lower BMD-TB as compared to non-carriers after 32 weeks (DSDS '0.92, p<0.01) and 1 year of therapy (DSDS '0.95, p<0.01). The MTHFR 1298 A>C SNP did not effect BMD values. Carriers of the MTRR 66 G-allele had a lower total body BMD during therapy as compared to non-carriers (DSDS '0.72, p<0.05). Carriers of both MTHFR 677T and MTRR 66G showed a decreased BMD-TB during treatment. Carriers of haplotype 3 of the VDR 5' Cdx-2/GATA polymorphism had a lower BMD-LS and/or BMAD compared with non-carriers after 32 weeks (DSDS '0.61, p<0.05 and DSDS '0.69, p<0.05), 1 year (DSDS '0.70, p<0.05 (BMD-LS)), 2 years of therapy (_SDS '1.15, p<0.01 and DSDS '1.18, p<0.01) and 1 year after cessation of therapy (DSDS '0.74, p<0.05 (BMAD)). Conclusion. No correlations were found between fracture risk and genotype. We identified the MTHFR C677T, MTRR A66G and VDR Cdx-2/GATA SNP's as determinants of treatment-related osteoporosis in pediatric patients with ALL.

0981

LATE RELAPSES IN T-ALL PATIENTS TRUE DISEASE RECURRENCE OR SECOND T-ALL?

T. Szczepanski,[1] V.H.J. van der Velden,[2] B. Gruhn,[3]
R. Panzer-Grümayer,[4] M. Spinelli,[5] H. Cavé,[6] D. Campana,[7]
A. Schrauder[8], E. van Wering[9], J.J.M. van Dongen[2]

[1]*Silesian Academy of Medicine, ZABRZE, Poland;* [2]*Erasmus MC, Rotterdam, Netherlands;* [3]*Friedrich-Schiller University of Jena, Jena, Germany;* [4]*Childrens Cancer Research Institute, Wien, Austria;* [5]*University of Padova, Padua, Italy;* [6]*Hpital Robert-Debr, paris, France;* [7]*St. Jude Children's Research Hospital, Memphis, USA; 8University Hospital Schleswig-Holstein, KIEL, Germany; 9Dutch Childhood Oncology Group, The Hague, Netherlands*

The vast majority of relapses in T-cell acute lymphoblastic leukemia (T-ALL) patients occur relatively early, usually within 2 years from diagnosis, frequently during maintenance treatment. Our previous comparative molecular analyses between diagnosis and relapse of such 'classical' T-ALL (26 patients) showed totally (62%) or at least partly (38%) identical T-cell receptor (TCR) gene rearrangement patterns at both disease phases. These results confirm that the relapse clone in these patients originated from the original diagnosis clone, which became resistant to the applied treatment. In contrast to these *classical* T-ALL, two patients experienced very late T-ALL recurrences (6 and 10 years from diagnosis, respectively) and both patients displayed completely different TCR gene rearrangement sequences between diagnosis and relapse. We hypothesized that such late *relapses* of T-ALL in fact might represent second malignancies and that patients developing such second leukemias might be genetically predisposed for T-ALL development. We succeeded to investigate 13 T-ALL patients with late relapses, i.e. at least 2.5 years from initial diagnosis. The studies at the DNA level involved detailed comparison of TCR gene rearrangements between diagnosis and relapse (PCR-heteroduplex, sequencing and/or Southern blot analyses) and the detection of gene fusions involving the TAL1 gene and/or TCR genes. We found the evidence of a common clonal origin between diagnosis and relapse in 8 of the 13 patients. In one case, the T-ALL had no clonal TCR rearrangements neither at diagnosis nor at relapse. Finally, in four patients TCR gene rearrangement sequences had completely changed between diagnosis and relapse, suggesting a second T-ALL rather than a relapse. We conclude that approximately 25% of late T-ALL 'relapses' in fact represent second malignancies. We are currently planning further genomic analyses to identify common genetic events or common genomic features which might be related to predisposition for development of T-ALL.

0982

RITUXIMAB AS A SINGLE AGENT DISPLAYS POTENT ACTIVITY AGAINST PRIMARY HUMAN CD20+ ACUTE LYMPHOBLASTIC LEUKEMIA IN A PRECLINICAL XENOGRAFTMODEL

B.A. Nijmeijer, M.L.J. van Schie, R. Willemze, J.H.F. Falkenburg

Leiden University Medical Center, Leiden, Netherlands

Monoclonal antibodies are emerging modalities in the treatment of hematologic malignancies. Rituximab (RTX), a chimeric antibody that recognizes CD20, shows therapeutic efficacy in non-Hodgkin lymphoma. Some precursor-B ALL (pB-ALL) express CD20. We evaluated the activity of RTX against primary human pB-ALL cells *in vitro*, as well as in a xenograft model of primary human pB-ALL. Eight out of 17 randomly selected pB-ALL samples revealed higher-than-baseline expression of CD20. In an *in vitro* complement mediated lysis (CDC) assay, RTX (10 μg/mL) induced significant lysis (>40%) in 8 out of the 17 samples. Lysis correlated significantly to CD20 expression (Pearson's correlation 0.89). CDC varied from 98±0.5% lysis in the sample with highest expression of CD20 to no lysis in CD20- samples. To evaluate activity of RTX against pB-ALL *in vivo*, NOD/scid mice were inoculated with primary CD20+ or with primary CD20-pB-ALL cells. Engraftment of leukemic cells was monitored by flow cytometric determination of leukemic cells in periodically taken blood samples. Upon emergence of leukemic cells, the treated group received four induction doses of 250 μg RTX with 24-hour intervals, followed by three weekly maintenance doses of 250μg RTX starting 7 days after induction. Control treated animals received a control antibody directed against CD25 which was not expressed by the pB-ALL cells. During treatment, periodical monitoring of blood samples was continued. Induction resulted in complete responses (CR) in the blood of all RTX -treated animals and these CR were maintained throughout the treatment period. In the control group, administration of the control antibody did not affect leukemic progression, nor did RTX affect leukemic progression in animals engrafted with CD20- pB-ALL cells. Significant plasma concentrations of RTX (range: 4.8 to 150μg/mL) could be detected in plasma of treated animals throughout the treatment period. At experimental end-point, animals were sacrificed and blood, spleen and bone marrow were analyzed for the presence of leukemic cells. In animals engrafted with CD20- pB-ALL cells and in all animals treated with control antibody, extensive infiltration of leukemic cells was observed (mean 65±12.8%, 35±1.8% and 77±12.9% in blood, spleen and marrow of all animals, respectively). In RTX-treated animals engrafted with CD20+ leukemia no leukemic cells could be detected in blood and spleen. However, infiltrates of leukemic cells were detected in bone marrow (mean 61±29.2%). Flow cytometric analysis revealed that these cells were saturated with RTX, indicating adequate in vivo exposure. The cells expressed lower levels of CD20 as compared to cells recovered from control treated animals but were still susceptible to RTX-induced CDC *in vitro*. Limited responses in the bone marrow may therefore have been due to limited potential of effector mechanisms in this compartment, rather than to loss of CD20 expression. In conclusion, our results suggest that RTX may have significant activity in CD20+ pB-ALL and clinical studies on the activity of RTX in CD20 positive pB-ALL are warranted.

0983

ETV6/RUNX1 DIRECTLY DYSREGULATES GENES WITH RUNX1 BINDING SITE VIA MECHANISM REVERSIBLE BY HISTONE DEACETYLASE INHIBITORS

J. Starkova,[1] J. Madzo,[1] M. Zaliova,[1] G. Cario,[2] A. Ford,[3] O. Hrusak[1] J. Trka[1]

[1]Childhood Leukaemia Investigation Prague, PRAGUE, Czech Republic; [2]University Med Center Schleswig-Holstein, KIEL, Germany;[3]LRFC, London, United Kingdom

RUNX1 is implicated in over 30 different translocations in human acute leukemia. *RUNX1*, can either activate or repress transcription of key regulators of cell growth and differentiation through binding to promoters or enhancer elements. The *ETV6/RUNX1* chromosomal translocation is the most common chromosomal aberration in paediatric cancers (25% of ALL). The ETV6 part of the fusion protein contains domains interacting with the mSin3, N-CoR and HDAC-3 corepressors. A part of the *RUNX1* gene involved in the fusion carries DNA-binding domain. *RUNX1* regulates haematopoietic myeloid cell differentiation and transcriptional activation but the role in lymphoid development is not yet fully understood. We hypothesize that *ETV6/RUNX1* causes pathological differentiation block in lymphoid cells. In the current project, we utilized treatment with histone deacetylase inhibitors (HDACi). We have previously confirmed specific effect of HDACi (valproate-VPA, Trichostatin A-TSA) on *ETV6/RUNX1* leukaemic cells in comparison with lymphoblastic leukaemias with different mechanism of leukaemogenesis (BCR/ABL and PDGFRα/ETV6). To prove the direct effect of HDACi on ETV6/RUNX1 *in vitro*, we utilized a target gene of *RUNX1*, granzyme B (GZMB). To determine whether *ETV6/RUNX1* represses GZMB via direct interaction with RUNX1-binding site at GZMB promoter, luciferase activity was measured in HeLa cells transfected with *pcDNA3.1-ETV6/RUNX1Myc* and compared with HeLa with pcDNA3.1 empty vector. Cells were transfected with pGZMB-luc or pGL3-basic to normalize the luciferase activity(pGZMB-luc/pGL3-basic). Fold change of ~3 FRU indicated that GZMB was downregulated by ETV6/RUNX1. To test the direct effect of HDACi on *ETV6/RUNX1*, after incubation of HeLa cells with VPA and TSA, luciferase activity was monitored again. Repression activity was reduced in treated transfected HeLa cells to 53% after VPA administration and 49% after TSA administration when compared to untreated cells. We used effect of HDACi on *ETV6/RUNX1* leukaemic cells and identified *ETV6/RUNX1* target genes in lymphoid cells. Analysis of expression profiling of treated (VPA, TSA) vs untreated (control) *ETV6/RUNX1*[+] REH cells showed genes with significantly changed expression after HDACi treatment. This group of genes was compared with a group of genes associated with *ETV6/RUNX1* phenotype selected by meta-analysis of expression data of ALL patients. Microarray data of selected genes showed downregulation of JunD, ACK1, PDGFRB in *ETV6/RUNX1*[+] patients as well as in our cell line model with increased expression after HDACi treatment. TCF4 gene was upregulated in the studied group and the administration of HDACi lead to its downregulation. Expression levels of chosen genes were validated by qRT-PCR: JunD - TSA p=0.013, VPA p=0.0008; PDGFRB - TSA p<0.0001, VPA p=0.016; TCF4 - TSA p<0.0001, VPA p=0.0002; ACK1 - VPA p=0.07. Selected genes have a fundamental role in cell proliferation and cell cycle progression therefore their role in leukaemogenesis is presumptive. We show for the first time direct transcription repression by ETV6/RUNX1 on GZMB gene model. These data also support our hypothesis that HDACi affect ETV6/RUNX1[+] cells via direct interaction with ETV6/RUNX1 protein, and that treatment with HDACi may release pathological differentiation block caused by ETV6/RUNX1 aberrant transcription factor.

Funding: Supported by grants IGA8316, GACR301/D189, GAUK75 and MSM0021620813.

0984

MINIMAL RESIDUAL DISEASE STATUS IS THE MOST IMPORTANT PREDICTIVE FACTOR IN ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA. PALG 4-2002 PROSPECTIVE ALL-MRD STUDY

J. Holowiecki,[1] M. Krawczyk-Kulis,[1] S. Giebel,[1] K. Jagoda[1], B. Stella-Holowiecka,[1] B. Jakubas,[2] M. Paluszewska,[3] I. Seferynska,[4] A. Czyz,[3] G. Palynyczko,[4] K. Nowak,[1] W. Baran,[3] A. Skotnicki,[2] W.W. Jedrzejczak,[5] K. Warzocha,[4] A. Hellmann[3]

[1]Silesian Medical University, Katowice, Poland; [2]Jagiellonian University, Krakow, Poland;[3]Medical Academy, Warsaw, Poland; [4]Blood Transfusion, Warsaw, Poland

Current therapeutic protocols for adult acute lymphoblastic leukemia (ALL) take into account the risk of relapse, in order adjust the treatment intensity to individual patient needs. It is postulated that in addition to classical risk criteria including age, cytogenetics, immunophenotype, and tumor burden, also minimal residual disease (MRD) should be considered for treatment decisions. The aim of this prospective study was to evaluate the feasibility and prognostic significance of MRD detected with the use of immunophenotyping for disease-free survival (DFS) of ALL patients treated according to 4-2002 protocol of the Polish Adult Leukemia Group (PALG). Induction therapy included prednisolone, asparaginase and 4x epirubicin+vincristin. Consolidation consisted of 2x high-dose AraC+cyclophosphamide, 2x methotrexate+etoposide, mercaptopurine, and CNS prophylaxis including irradiation. Patients stratified to high risk group according 'classical' criteria based on those formerly developed by GMALL (bcr/abl(+), WBC>30 G/L, prepreB or preT phenotype, age>35 years, or 2 courses of induction required to achieve CR) were further referred for bone marrow transplantation, whereas those assigned to standard risk group (none of the above factors present) were treated with maintenance for two years. MRD was tested at the level of 0.1% after completion of induction and consolidation therapy in patients achieving CR, employing multicolor flow-cytometry. For patients with specific antigen combinations a standard *quadrans* method was used, for the remaining ones we applied a new *empty spaces* method taking into account an individual antigen expression on blast cells. The *forbidden* gates were established with the use of triple staining by comparison with the pattern obtained for healthy volunteer bone marrow donors. At least two antigen combinations were tested for each patient. One-hundred-ten ALL patients (B-lineage 82%, T-lineage 17%), aged 30 years (17-61) treated in 16 hematological centers were included in the analysis. CR rate equaled 80%. Among patients who achieved CR, 24% were assigned to standard risk, 76% - to high risk group, according to classical criteria. MRD evaluation was possible in all CR patients. In 50% of patients MRD was negative after both induction and consolidation - MRD($^-$) group, whereas in the remaining 50% of cases MRD was detected at least once - MRD($^+$) group. At 3 years the probability of DFS in MRD($^-$) and MRD($^+$) group equaled 58% and 28%, respectively (p=0.04). The prognostic value of MRD status for DFS was more pronounced in patients with standard risk ALL: 80% for MRD(-) vs. 0% for MRD($^+$)(p=0.048), than in those with high risk ALL: 51% vs. 33%, respectively (p=0.23). In a multivariate analysis including classical prognostic criteria the MRD status remained the only significant predictive factor (HR: 1.33 (1.24-22.46), p=0.04). We conclude that immunophenotyping employing *empty spaces* method is feasible for MRD evaluation in adults with ALL. MRD status after induction and consolidation is the most important predictive factor for DFS. In particular, patients assigned to standard risk according to classical criteria can be further stratified and those with MRD detected after induction and/or consolidation should be offered intensified treatment with the use of hematopoietic cell transplantation.

Hodgkin's Lymphoma - Clinical Trials

0985

RECENT INTERIM ANALYSIS OF THE HD11 TRIAL OF THE GHSG: INTENSIFICATION OF CHEMOTHERAPY AND REDUCTION OF RADIATION DOSE IN EARLY UNFAVORABLE STAGE HODGKIN'S LYMPHOMA

A. Engert,[1] V. Diehl,[2] C. Brillant,[2] A. Engert,[2] R.P. Mueller,[3] H.T. Eich,[3] K. Mueller-Hermelink,[4] R. Herrmann,[5] J. Markova,[6] H. Ho,[7] W. Hiddemann[8], B. Doerken[9], R. Greil[10], A. Josting[1], B. Pfistner[2]

[1]*University Hospital of Cologne, Koln, Germany;* [2]*Clinic I of Internal Medicine, GHSG, Cologne, Germany;* [3]*Dept. of Radiotherapy, University Hospit, Cologne, Germany;* [4]*University of Wuerzburg, Wuerzburg, Germany;* [5]*Dept. of Oncology, Kantonsspital Basel, Basel, Switzerland;* [6]*University Hospital of Prague, Prague, Czech Republic;* [7]*University of Heidelberg, Heidelberg, Germany; 8University Hospital Grohadern, München, Germany; 9University Hospital Berlin, Berlin, Germany;* [10]*University of Innsbruck, Innsbruck, Austria*

Backgrounds. Combined modality treatment consisting of chemotherapy (CT) followed by involved field radiotherapy (IF-RT) is the standard treatment for early unfavourable Hodgkin's lymphoma (HL). Despite high complete remission (CR) rates, failures are common. We thus compared the baseline-dose BEACOPP regimen with ABVD and 20 with 30 Gy IF-RT in a prospectively randomized trial (HD11) in an attempt to improve outcome in this group of patients. *Methods.* Between May 1998 and January 2003, 1570 patients (pts) aged 16-75 with untreated intermediate stage HL (CS I, IIA with risk factors or IIB with elevated ESR and/or ≥ 3 nodal areas only) were randomized according to a factorial design between 4 cycles of ABVD followed by 30 Gy IF-RT (arm A - standard treatment), 4 ABVD + 20 Gy IF-RT (arm B), 4 baseline-dose BEACOPP + 30 Gy IF-RT (arm C) and 4 baseline-dose BEACOPP + 20 Gy IF-RT (arm D). *Results.* In the fifth preplaned interim analysis, 1293 pts were evaluable for the chemotherapy comparison and 1274 for the radiotherapy comparison. Patient characteristics were well balanced between the treatment arms. 95% of patients treated reached CR, 2% had pogressive disease, 8% relapsed and the total mortality rate was 4% with no significant differences between treatment arms for either endpoint. The most frequent haematological toxicities during chemotherapy were leucopenia observed in 32% of pts (ABVD: 25%, BEACOPP: 39%) and anemia in 4% of pts (ABVD <1%, BEACOPP 7%). Infection rate was 5% (ABVD 3%, BEACOPP 7%). The most frequent toxicity during radiotherapy was dysphagia in 5%. 14 secondary neoplasias were observed: 2 AML, 4 NHL, 8 solid tumors with no significant differences between treatment arms. After a median observation time of three years, freedom from treatment failure (FFTF) was 87% (95%-CI 85-89) and overall survival (OS) was 96% (95%-CI 95-97). Both for FFTF and OS, there was no sequential significant difference either between ABVD (FFTF 87%, OS 97%) and BEACOPP (FFTF 88%, OS 96%) nor 30 Gy (FFTF 90%, OS 97%) and 20 Gy IF-RT (FFTF 87%, OS 97%). *Conclusions.* At three years of median observation time, no sequential significant differences in treatment outcome were detected, neither between chemotherapy regimens nor between the different doses of radiotherapy, despite more relapses in 20 Gy radiotherapy arms.

0986

COMBINED MODALITY TREATMENT OF TWO OR FOUR CYCLES OF ABVD FOLLOWED BY INVOLVED FIELD RADIOTHERAPY IN THE TREATMENT OF PATIENTS WITH EARLY STAGE HODGKIN'S LYMPHOMA: UPDATE INTERIM ANALYSIS OF THE RANDOMISED HD10 STUDY OF THE GERMAN HODGKIN STUDY GRO

A Engert,[1] A. Pluetschow,[2] H.T. Eich,[3] R. Herrmann,[4] B. Doerken,[5] L. Kanz,[6] R. Greil,[7] J. Markova[8], B. Pfistner,[2] A. Josting[1], K. Mueller-Hermelink[9], R.P. Mueller,[3] V. Diehl[2]

[1]*University Hospital of Cologne, KLN, Germany;* [2]*Clinic I of Internal Medicine, GHSG, Cologne, Germany;* [3]*Dept. of Radiotherapy, University Hospit, Cologne,, Germany;* [4]*Dept. of Oncology, Kantonsspital Basel, Basel, Switzerland;* [5]*University Hospital Berlin, Berlin, Germany;* [6]*University of Tuebingen, Tuebingen, Germany;* [7]*University of Innsbruck, Innsbruck, Austria; 8University Hospital of Prague, Prague, Czech Republic; 9University of Wuerzburg, Wuerzburg, Germany*

Background and Aim. Combined modality treatment is regarded as standard by most study groups for patients with early-stage Hodgkin's lymphoma (HL). However, the optimal chemotherapy, the number of cycles needed and the optimal radiotherapy dose is still unclear. The GHSG thus conducted a randomised study for patients with early stage favourable Hodgkin's lymphoma (HD10) in which these questions were addressed. *Methods.* A total of 1370 patients were randomised from 5/1998 to 1/2003 between two or four cycles of ABVD and independently to 20Gy or 30Gy involved field (IF) radiotherapy. *Results.* For the second interim analysis at a median follow up of 28 months, 847 patients were available. Patients were equally balanced for age, gender, stage, histology, performance status and risk factors. Compared with two cycles, there was more toxicity in patients receiving four cycles of ABVD for leucopenia, hair loss and infection. Concerning radiotherapy dose, there was more toxicity associated with 30Gy for dysphagia, mucositis and leucopenia. The rate of complete remissions ranged between 98% and 99% with no significant differences among treatment arms. Freedom from treatment failure (FFTF) and overall survival showed no differences between the four treatment arms. The curves for overall survival and FFTF were nearly superimposable for all four arms. *Conclusion*. This analysis suggests that 2 chemotherapy cycles with involved field radiotherapy may be sufficient for patients with early favourable HL, but a reliable assessment must await the final analysis including all randomised patients and with adequate follow-up. The results of the third interim analysis (10/2005) including 1110 patients with a median follow up of more than 3 years will be presented.

0987

PREDICTIVE VALUE ON TREATMENT OUTCOME OF EARLY [18F]-FDG PET SCAN IN ADVANCED STAGE HODGKINS DISEASE TREATED BY CONVENTIONAL CHEMOTHERAPY IS SUPERIOR TO IPS SCORE

A. Gallamini

Azienda Ospedaliera S. Croce e Carle, Cuneo, Italy

Backgrounds. FDG-PET scan performed early during therapy (ChT) is a powerful prognostic tool in lymphomas. Aims. Starting in January 2002, 132 new, advanced-stage HD pts, consecutively admitted to twelve Italian hematological institutions were enrolled in a prospective multicenter clinical trial aimed at comparing the predictive value on treatment outcome of International Prognostic Score (IPS) with FDG-PET scan performed after two courses of ABVD in untreated advanced stage HD patients (pts). *Patients*. The mean age was 33.6 years (14-79), the male to female ratio 65/67; advanced disease (stages IIB-IVB) was present in 94, and stage IIA with adverse prognostic factor (> 3 nodal sites involved, sub-diaphragmatic presentation, bulky disease and ESR > 40) in 38. Bulky and extra-nodal disease were recorded in 47 and 40 pts, respectively. All pts were staged at baseline, after 2 courses of ChT and at the end of treatment by CT scan and FDG-PET scan (CT0, PET-0; CT-2, PET-2 and CT-6, PET-6, respectively). The mean interval between the end of the second ChT course and PET-2 was 11.6 days (2-32); the interval between the end of the therapy (including radiotherapy) and PET-6 was never shorter than 50 days. 126/132 pts. were treated with ABVD x 6; 6 by COPP/EBV/CAD x 6. At the end of ChT in 66/132 pts. with bulky disease consolidation radiotherapy was given. All patients were given the therapy programmed at baseline, except in case of overt progression. *Results*. The mean follow-ups from the diagnosis and from final restaging were 609 days (73-1513) and 402 days (0-1240), respectively. 108 pts attained CR while 24 were chemoresistant: 19 showed disease progression during therapy 1 was RP and 4 showed early relapse (within 6 months) after CR entry: (+28 - +178 days). One out of the 108 pts attaining CR showed a late relapse 18 months after CR entry. In univariate analysis, besides PET-2 (p<0.01), the clinical factors that were significantly associated with a higher probability of treatment failure were stage (p<0.01), International Prognostic Score (p< 0.01), WBC (p<0.01), Extra-nodal sites (p<0.01). The only factor independently significant for relapse/progression probability in multivariate analysis was PET-2, with a very high hazard ratio (60.9; 95% C.I. 17.9 - 207.0). In terms of treatment failure, the Positive Predictive Value (PPV) of a PET-2 and IPS (Score 0-2 vs 3 or more) were 88% and 41% and the Negative Predictive Value (NPV) were 98% and 88%, respectively. The sensitivity of PET-2 and IPS were 92% and 46%, the specificity were 97% and 85% and the overall accuracy 96% and 78%, respectively. The 2-y FFS and PFS probability for PET-2 negative and for PET-2 positive patients were 97% and 97% and 7% and 18%, respectively (FFS Log Rank test = 135.1, p<0.01; PFS log rank test=114.0, p<0.01). Conclusions. PET-2 scan is the most powerful tool so far available for predicting treatment outcome in advanced-stage HD.

0988

AMH AND INHIBIN B ARE VALUABLE NEW MARKERS FOR GONADAL DAMAGE AFTER THE TREATMENT OF M.HODGKIN WITHOUT RADIOTHERAPY

R.D. van Beek,[1] S.M.P.F. de Muinck Keizer-Schrama,[1] R. Weber,[2] J.S.E. Laven,[2] F.H. de Jong,[2] F.G. Hakvoort-Cammel,[1] C. van der Bos,[3] R. Pieters,[1] M.M. van der Heuvel[1]

[1]*Erasmus MC-Sophia, Rotterdam, Netherlands;* [2]*Erasmus MC, Rotterdam, Netherlands;* [3]*AMC, Amsterdam, Netherlands*

Background. An important long-term effect of both radiotherapy and chemotherapy is gonadal dysfunction. Aim of this study is to evaluate the gonadal long-term effects of the treatment for childhood M. Hodgkin (HD) with combination chemotherapy (ABVD or EBVD with/without MOPP) and to identify markers for long-term follow-up of gonadal function. Methods. Eighty-six pediatric HD patients treated from 1974-1998 were included. All patients were in complete remission. Median follow-up was 15.5 yr. (range 5.6-30.2 yr.), median age at follow-up was 27.0 yr. (range 17.7-42.6 yr.). Follicle stimulating hormone (FSH), luteinizing hormone (LH) and inhibin B were determined in all patients. Additionally, in men testosterone and sex hormone binding globuline (SHBG) and in women 17 β-estradiol and anti-Müllerian hormone (AMH) were determined. In 20 men semenanalyses were performed. Results. In men treated with MOPP median FSH (16.6 U/l vs. 2.4 U/l; $p<0.001$) and LH (5.7 U/l vs. 2.5 U/l; $p<0.001$) were significantly increased as compared to patients treated without MOPP. Inhibin B (17.5 ng/l vs. 143 ng/L; $p<0.001$) and semen concentration (1.1*10×6/mL vs.49.5*10×6/mL; $p<0.05$) were significantly decreased. Inhibin B was strongly correlated with semen concentration (rs=0.83; $p<0.001$). FSH (rs=0.68; $p<0.001$) and inhibin B (rs=-0.68; $p<0.001$) were correlated with cumulative dose procarbazine. In women no significant differences in LH, FSH, inhibin B or estradiol between patients treated with or without MOPP were found, but AMH was significantly lower in patients treated with MOPP as compared to patients treated without MOPP (0.39 μg/L vs. 1.40 μg/L; $p<0.01$). AMH levels were correlated with cumulative dose procarbazine (rs=-0.54; $p<0.01$). *Conclusion*. This study shows that AMH and inhibin B are valuable new serum markers for gonadal damage after pediatric HD. In men inhibin B is strongly correlated with semen concentration, whereas in women AMH detects early gonadal damage even in cases with normal LH/FSH levels.

0989

THE POLYMORPHISM IN THE INTERLEUKIN-10 GENE PROMOTER AT -592 IS A PROGNOSTIC MARKER IN HODGKIN'S LYMPHOMA

S. Hohaus, M. Giachelia, A. Di Febo, M. Martini, G. Massini, B. Vannata, G. Mansueto, S. Piano, F. Guidi, F. D'Alo', L.M. Larocca, M.T. Voso, G. Leone

Università Cattolica S. Cuore, Rome, Italy

Backgrounds. Hodgkin's lymphoma is characterized by an abundant immune infiltrate surrounding the malignant Reed-Sternberg cells, and it is thought that the production of cytokines contributes to this abnormal immune response. Single nucleotide polymorphism in the 5'-promoter region of cytokine genes are key factors for cytokine production and may modify the biology of the disease. Recently, differences in the prognosis according to the Interleukin-10 (IL-10) genotype have been shown in patients with diffuse large B cell lymphomas (*Lech-Maranda et al, Blood 2004; 103:3529*). *Aim.* To assess the role of polymorphisms in the Interleukin-10 gene on progression-free survival in Hodgkin's lymphoma. *Methods.* We assessed the distribution of frequencies of polymorphic allele variants in the IL-10 gene (T-3575A, G-2849A, C-2763A, A-1082G and C-592A) in 204 patients with Hodgkin's lymphoma and analysed for associations with patient charcteristics and prognosis. The polymorphism were analyzed using a multiplex amplification and mismatched polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). DNA was extracted either from peripheral blood or paraffin-embedded lymph node biopsies from 204 patients with Hodgkin's lymphoma (median age 32 years, range 14-77 years; 91 females and 113 males). 194 patients were treated with standard chemotherapy regimens: 115 patients received ABVD, 34 pts a modified Stanford V regimen (substituting 6 mg/m^2 metchloramine with 650 mg/m^2 cyclophosphamide), 24 pts MOPP (+ABVD), 21 pts BEACOPP. The prognostic role of allelic variants were analyzed as SNPs, and of haplotypes which were reconstructed using the PHASE programme. *Results.* The distribution of allele frequencies in Hodgkin's lymphoma at position -592 of the IL-10 gene was as follows: 46% were homozygous for the CC genotype, 40% were heterozygous and 14% were homozygous for the AA genotype. The IL10 -592AA genotype was associated with a decreased progression-free survival ($p=0.0074$). The probability of progression-free survival at a median time of observation of 4 years for patients homozygous for the IL-10 -592 AA genotype was 33% (95% C.I, 14-54%), while for heterozygous patients and for patients homozygous for the -592 C allele it was 70 and 74% (95% C.I., 56 -80, and 61-83), respectively. When the analysis was restricted to 115 patients treated with ABVD chemotherapy, essentially the same differences in progression-free survival were observed. In univariate analysis of established prognostic factors, stage proved to be of prognostic value in our patient group (limited disease in stage I-IIA vs advanced disease in stage IIB-IV, $p=0.013$) The Cox multivariate analysis showed that IL-10 -592AA genotype and stage were independent prognostic factors ($p=0.02$ and 0.016, respectively). *Conclusion*. Our study indicates that the IL-10 genotype can predict clinical outcome in patients with Hodgkin's lymphoma and points to the importance of the genetic background of the host.

Cell signaling, transcriptional control and apoptosis - II

0990

CD95L EXPRESSING ANTIGEN-PRESENTING CELLS PREVENT ANTIGEN-SPECIFIC T CELL RESPONSE BY APOPTOSIS INDUCTION AND INHIBITION OF T CELL ACTIVATION

G. Strauss, H. Kasperczyk, S. Fulda, K.M. Debatin

University Children's Hospital, ULM, Germany

Background. Selective depletion of antigen-specific T cells e.g. through induction of apoptosis via the CD95 system may have widespread applications in transplantation settings or in the treatment of autoimmune diseases. Antigen presenting cells (APC) expressing death-inducing ligands such as CD95 ligand (CD95L) could theoretically be used as immunomodulators in an antigen-specific counterattack system. Since human naive T cells are resistant to CD95-mediated apoptosis and acquire CD95-sensitivity only after activation, CD95L expressing APC might selectively deplete antigen-specific T cells while leaving naïve T cells untouched. Aims. We studied the modulation of an alloimmune response and changes in T cell activation in the presence of CD95L-expressing APC. *Methods.* The HLA-A1 expressing lymphoblastoid cell line C1R.A1 was transfected with membrane-bound CD95L (m-CD95L), which was stably expressed on the cell surface of the APC due to a mutation in the metalloproteinase cleavage site. HLA-A1 negative T cells were stimulated with m-CD95L expressing C1R.A1 cells or with a mock transfectant to study the development of the HLA-A1 specific alloimmune response. Results. m-CD95L expressing APC were able to induce apoptosis in CD95 expressing activated primary T cells. Constitutive presence of m-CD95L in the stimulation cultures inhibited the development of $CD4^+$ and $CD8^+$ HLA-A1-specific T cells. However, immunity towards third-party, viral, and bacterial antigens was maintained and T cells spared from depletion could be induced to develop cytotoxicity towards unrelated antigens. Interestingly, inhibition of HLA-A1 specific T cell response absolutely requires the co-expression of m-CD95L and HLA-A1 antigen on the same APC. The simultaneous analysis of proliferation and apoptosis induction in HLA-A1 negative T cells activated with m-CD95L expressing APC indicated that activated T cells are depleted by cell death induction while proliferation of naïve T cells was inhibited. naïve T cells activated by m-CD95L expressing APC exhibited a reduced expression of activation markers (CD25, CD69, CD71, HLA Cl II) and Th1 and Th2 cytokines. Ca influx was diminished when cells were stimulated by CD95L expressing APC compared to the mock transfectant. However, differences in NF-kB activation were not observed independent whether m-CD95L was absent or present. Efficiency of inhibition of T cell activation by m-CD95L expressing APC was dependent on the expression level of m-CD95L. *Conclusions*. m-CD95L expressing APC represent efficient immunomodulators to achieve antigen-specific tolerance since they simultaneously induce apoptosis in activated T cells and prevent T cell activation of naive T cells without impairment of immune responses towards unrelated antigens.

0991

THE PROTO-ONCOGENE EVI1 INDUCES FETAL ANEMIA IN A CONDITIONAL TRANSGENIC MOUSE MODEL

H.C.M. van den Akker,[1] D. Spensberger,[2] K. van Lom,[2] A. van Hoven-Beijen,[2] C. Erpelinck,[2] R. Ferreira,[3] U. Klingmller,[4] J.N.J. Philipsen,[3] J. Downing[5]

[1]*Erasmus MC, Rotterdam, Netherlands;* [2]*Department of Hematology, Erasmus MC, Rotterdam, Netherlands;* [3]*Department of Cell Biology, Erasmus MC, Rotterdam, Netherlands;* [4]*German Cancer Research Center, Heidelberg, Germany;* [5]*St.Jude Children's Research Hospital, Memphis, USA*

Background. Aberrant expression of the proto-oncogene EVI1 has been observed in patients with acute myeloid leukemia, chronic myeloid leukemia or myelodysplastic syndrome carrying 3q26 aberrations. Patients with high EVI1 expression respond poorly to anti-leukemic therapy. Although it is generally believed that *EVI1* transforms hematopoietic stem cells, there is evidence that *EVI1* may interfere more directly with myeloid or erythroid development, and lineage-specific effects may play a role in the pathogenesis of leukemia or myelodysplastic syndromes. *Aims*. Since MDS with 3q26 abnormalities and aberrant *EVI1* expression is characterized by severe anemia, our aim was to determine the direct effects of *EVI1* on erythropoiesis *in vivo*. Moreover, our approach allowed us to investigate the effects of *EVI1* when expressed at different stages of erythroid differentiation. *Methods*. To prevent the embryonic lethality associated with conventional EVI1 transgenic models and to allow the study of effects of EVI1 in separate hematopoietic lineages, we established transgenic mouse lines with conditional, Cre-inducible hematopoietic expression of EVI1. In these Vav-LSL-EVI1 transgenic mice, EVI1 transcription is blocked by the presence of a loxP-flanked transcriptional stop sequence (LSL). The EVI1 transgenic lines were crossed with two different erythroid lineage specific Cre transgenic lines to specifically induce EVI1 expression at different stages of erythroid differentiation. The EpoR-Cre and pEV-Cre transgenic lines express Cre from the BFU-E and CFU-E stage onward, respectively. *Results*. Erythroid-specific EVI1 overexpression induced fetal anemia, with major defects in primitive and definitive erythropoiesis. Fetal livers from both VAV-LSL-EVI1/pEV-Cre and VAV-LSL-EVI1/EpoR-Cre double transgenic animals were small, pale and contained decreased cell numbers as compared to livers from single transgenic or wild type littermates. However, the phenotype in VAV-LSL-EVI1/EpoR-Cre embryos was clearly more severe. Colony assays demonstrated that VAV-LSL-EVI1/EpoR-Cre transgenic fetal livers contained less BFU-E and CFU-E erythroid progenitors, while in VAV-LSL-EVI1/pEV-Cre embryos only CFU-E numbers were reduced. Moreover, a more complete block in terminal erythroid differentiation and a more profound increase in the number of apoptotic and dysplastic fetal liver erythroid cells were observed in VAV-LSL-EVI1/EpoR-Cre embryos. *Ex-vivo* experiments suggest that the EVI1-induced embryonic lethality in VAV-LSL-EVI1/EpoR-Cre as opposed to Vav-LSL-EVI1/pEV-Cre mice may be due to a reduced sensitivity of VAV-LSL-EVI1/EpoR-Cre erythroid cells to respond to Epo. *Summary/Conclusions*. Our results show that EVI1 directly interferes with the survival, expansion and differentiation of erythroid progenitors in vivo, and that the severity of the defects increases when EVI1 is induced at an earlier stage of erythropoiesis. We have established a conditional EVI1 transgenic mouse model that in combination with other inducible or lineage specific Cre-lines, e.g. Mx1-Cre can be applied to study the involvement of EVI1 in MDS and AML.

0992

ROLE OF LYMPHOID MICROENVIRONMENT IN INHIBITION OF APOPTOSIS AND ACTIVATION OF PI3-K/AKT PATHWAY AND PTEN IN B-CLL CELLS

M. Shehata, S. Schnabl, D. Demirtas, J. Schwarzmeier, M. Hilgarth, R. Hubmann, M. Duechler, A. Gaiger, U. Jäger

Medical University of Wien, Wien, Austria

Background. The accumulation of the malignant B cells in chronic lymphocytic leukemia (B-CLL) appears to be due to inhibition of apoptosis and long survival of the leukemic cells. This could be due to the activation of anti-apoptotic mechanisms in the leukemic cells through their interaction with the lymphoid microenvironment. *Aim.* The aim of this study is to elucidate the role of the lymphoid microenvironment in activation of the potent anti-apoptotic PI3-K signal transduction pathway and prolongation of survival of B-CLL cells. *Methods*. Stromal fibroblasts of bone marrow (BMF), spleen (SF) and lymph gland (LGF) were used as an *in vitro* model for lymphoid microenvironment and to test their ability to inhibit spontaneous apoptosis of B-CLL. Pharmacological inhibitors and siRNAs against PI3-K and Akt were applied to explore the anti-apoptotic effect of this pathway in B-CLL. *Results*. Co-cultivation of B-CLL cells with human BMF, LGF, and SF significantly inhibited apoptosis and prolonged survival of the leukemic cells in comparison to suspension cultures and to co-cultures with fibroblasts from non-lymphoid organs. Trans-well culture experiments indicated that cell-cell interaction and soluble mediators are essential for this supportive effect. To explore the involvement of PI3-K/Akt pathway in the anti-apoptotic effect of stromal fibroblasts, co-cultures were performed in presence of PI3-K inhibitors (wortmannin or LY294002) or siRNAs against PI3-K (p110-β subunit) and Akt1. These inhibitors significantly reduced the supportive effect of stromal fibroblasts and induced apoptosis in B?-CLL cells. Interestingly, the leukemic cells were far more sensitive to PI3-K inhibition than T cells, monocytes and fibroblasts. Induction of apoptosis was associated with a significant decrease in the intracellular PIP3, PI3-K, PDK1 and Akt1, NF-κ B, IKK, and dephosphorylation (activation) of tumour suppressor protein PTEN. Studies using phosphospecific anti-PTEN antibody demonstrated that PBMC of CLL patients (n=40) highly express a phosphorylated (inactive) form of PTEN. *Conclusion*. The results demonstrate that PI3-K/Akt pathway is involved in inhibition of apoptosis of B-CLL cells and suggest that interaction of the leukemic cells with lymphoid microenvironment maintains the activation of this pathway. The data also suggest that targeting this pathway represents a new therapeutic approach in B-CLL.

0993

ANALYSIS OF APOPTOSIS SIGNALING IN PRIMARY LEUKEMIA CELLS AND ITS IMPACT ON TREATMENT RESPONSE AND LONG TERM SURVIVAL

K. Stahnke, L.H. Meyer, S.M. Eckhoff, K.M. Debatin

University Childrens Hospital, Ulm, Germany

Drug resistance and treatment failure in acute leukemia has been attributed to apoptosis resistance in leukemia cells as defects in apoptosis signal transduction are commonly acquired during malignant transformation. However, expression analysis of apoptotic molecules with regard to clinical outcome has so far failed to identify apoptosis defects with prognostic value. Since the efficacy of apoptosis signaling is probably not sufficiently represented by the expression of apoptosis molecules alone, we developed and evaluated different assays to assess the function of apoptotic pathways in primary leukemia cells. Flow cytometric quantification of caspase activation by cleavage of the rhodamine derivative (Z-DEVD)2R revealed a broad variation in the extent of caspase-3 activity in primary pediatric B-precursor ALL cells upon cultivation in medium, which might be of prognostic relevance. Despite similar induction of cell death, a differential activation of caspase-3 by Cytarabine and Cyclophosphamide could be assessed, indicating drug specific differences in activation of apoptosis signaling. In a xenotransplant disease model for pediatric ALL, drug induced caspase activation could be quantified, demonstrating its potential use for monitoring drug efficacy *in vivo*. In order to test the functional integrity of a core apoptosis signaling pathway, we have developed and evaluated a method for the simultaneous measurement of two apoptogenic events in individual cells: caspase-3 activation and cytochrome c release, using conformation sensitive monoclonal antibodies. This method proved to identify deficient mitochondrial apoptosis signaling in leukemia cells overexpressing Bcl-2 by a pattern of apoptosis resistance, deficient cytochrome c reduction and partial processing of caspase-3. By combination of these techniques, we were able to analyze and, more importantly, to quantify potential defects in apoptosis signal transduction on a single cell level in patient samples cultured *in vitro*. We analyzed the activation of apoptosis signaling in primary leukemia cells during apoptosis induction by the physiologic stimulus of lack of survival factors in order to identify constitutive defects in apoptosis signaling in individual leukemia samples. Activation and mutual correlation of cytochrome c release and caspase-3 activation was quantified in 78 patient samples of precursor B-cell ALL. We identified a novel parameter, CRAC (Cytochrome c - Related Activation of Caspases 3) reflecting proficient or deficient cytochrome c related caspase activation in the individual patient sample with prognostic impact on treatment failure and relapse. At a median follow-up of 31 months, disease-free survival was 84 months (95% CI = 76 to 91 months) and 66 months (95% CI = 52 to 80 months) for patients with positive and negative CRAC respectively (p=0.019). CRAC may thus serve as a functionally defined risk factor for treatment stratification. Functional analysis of apoptosis signaling in primary leukemia may help to identify molecular targets for improvement of anti-leukemic treatment.

0994

VEGF REGULATES LEUKEMIA MIGRATION VIA FLT-1, INVOLVING PI3 KINASE, RHOA AND RAC1 ACTIVATION AND LIPID RAFTS/CAVEOLAE FORMATION

R. Fragoso, C. Casalou, S. Dias

Portuguese institute of Oncology, Lisboa, Portugal

Vascular endothelial growth factor (VEGF) and its receptors play a crucial role in malignancy and in disease, regulating the survival, proliferation, and migration of several cell types, such as endothelium and also leukemia cells. We previously demonstrated crucial roles for VEGFR-2 in acute myeloid leukemia, where its blockade showed clinical potential in murine models, by affecting leukemia survival and proliferation. In the present study we focused on a different VEGF receptor, FLT-1, and studied the molecular mechanisms whereby it modulates acute leukemia cell migration in response to VEGF/Placental Growth Factor (PLGF). First, we observed the formation of cell protrusions on ALL cells after VEGF/PLGF stimulation, with evidence for polimerized actin and FLT-1 co-localization (as determined by phalloidin, immunofluorescence staining, and confocal microscopy). Western blot analysis revealed that PLGF/VEGF stimulation resulted in increased RhoA and Rac1 GTPases expression. Co-treatment with LY200942 significantly decreased RhoA and Rac1 induction and cell migration by PLGF/VEGF, demonstrating this effect is modulated via Pi3 kinase. Next, we investigated the mechanisms whereby FLT-1 and actin co-localize at the cell 'leading edge' (protrusions), after VEGF/PLGF stimulation, and the relevance of such co-localization for cell migration. We addressed this question by impairing the formation of lipid rafts/caveolae using drugs whether to sequestering (nystantin) or depleting (methy-β-ciclodextrin) cholesterol. Accordingly, co-treatment of leukemia cells with nystantin/methyl-β and PLGF/VEGF blocked cell migration, an effect that was associated with a decrease in FLT-1 polarization and co-localization with actin filaments. Instead, FLT-1 was now found mostly in the cell cytosol (possibly undergoing lysosomal degradation). Taken together, we hypothesize that FLT-1 localization in lipid rich membrane domains allows interaction with the actin cytoskeleton and downstream effectors, resulting in cell migration. Our data reveal for the first time some of the molecular mechanisms involved in VEGF-mediated leukemia migration, which may be crucial for determining the onset of extramedullary disease (the exit of leukemia cells from the bone marrow).

Anemia/Red blood cells

0995

COLD SHOCK DOMAIN PROTEIN A (CSDA) ACTS AS REPRESSOR FACTOR OF γ GLOBIN GENE EXPRESSION *IN VIVO*

M.G. Grosso

University of Naples Federico II, Naples, Italy

Impaired hemoglobin switching leading to persistent expression of fetal globin genes in adults (HPFH) offers great therapeutic potential for hemoglobinopathies and much effort is underway to clarify the molecular basis of this mechanism.[1] In order to identify and study regulatory factors putatively involved in γ-globin gene expression, we examined the reticulocyte mRNAs differently expressed in three siblings presenting different levels of HbF and varied severity of β-thalassemia intermedia conditions, even though sharing the same α- and β-globin gene cluster genotypes. In fact, all of them showed the homozygous state for the β+ IVSI-6 (C'T) mutation associated to haplotype VI chromosomes and a normal set of α-globin genes. To investigate the possible causes of the variations in γ-globin gene expression, extensive sequence analysis was performed on putative regulatory regions within the β-globin gene cluster.[2] Results showed the same genetic background in all the siblings and excluded HPFH mutations. It was thus supposed that genetic determinants not linked to the β-globin gene cluster were responsible of the different γ-globin gene expression levels. To explore this hypothesis, the reticulocyte transcriptome was analyzed by a differential mRNA display approach, revealing several bands differentially displayed in the sample from the brother respect to his sisters. Selected bands were cloned and sequenced. A complete homology (greater than 95%) with the cDNA sequence of the cold shock domain protein A (CSDA), acting as repressor factor for several hematopoietic genes and previously reported to be able to interact with the γ-globin gene promoter[3,4] was found for two of the clones originated from bands with increased expression in the brother. Quantitative real time PCR analysis of CSDA and γ-globin gene mRNA levels was performed on reticulocyte RNAs to confirm data obtained by differential display and revealed an inverted correlation between HbF values and CSDA mRNA levels, comparable to that found between CSDA and γ-globin gene mRNAs. To analyze the role played by CSDA in regulating the expression of γ-globin genes, transient RNAi was used to elicit its knockdown in K562 cell line. Results showed a two-fold increased level of γ-globin mRNA when CSDA expression was interfered at about 40-50%. CSDA has been previously reported to interact with the -200 promoter region of the γ-globin gene where some HPFH mutations fall[4] and a possible mechanism of trans-acting regulation of γ-globin gene expression has been proposed. Our data, in agreement with this hypothesis, provide further insights into the involvement of CSDA in the control of γ-globin genes expression. In fact, in our case, no HPFH mutations were detected but it is rather conceivable, on the basis of RNAi results, that a quantitative defect of CSDA expression may produce a significant persistence of HbF in adult life, thus suggesting possible novel targets for gene therapy in hemoglobinopathies.

References

1. S.L. Thein. Br. J. Haematol 2004; 124: 264-74
2. Zhi-Hong Lu et al., Blood 1996; 87: 1604-11
3. P. Coles et al. Nucleic Acids Res 1996; 12: 2311-17
4. Horwitz M. et al. JBC 1994; 269: 14130-39

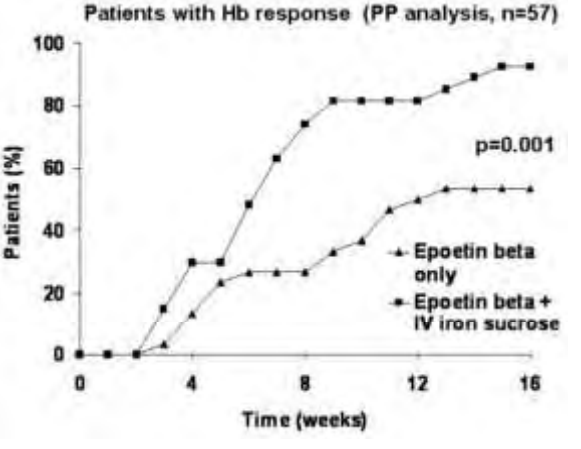
0996

ADJUVANT INTRAVENOUS IRON THERAPY POTENTIATES EPOETIN β TREATMENT IN ANEMIC, NON IRON-DEPLETED PATIENTS WITH LYMPHOPROLIFERATIVE DISORDERS: RESULTS OF THE NIFE STUDY

M. Hedenus,[1] G. Birgegård,[2] P. Näsman,[3] L. Ahlberg,[4] T. Karlsson,[8] B. Lauri,[5] J. Lundin,[6] G. Lärfars,[7] A. Österborg[6]

[1]*Sundsvall Hospital, SUNDSVALL, Sweden;* [2]*Akademiska Hospital, UPPSALA, Sweden;* [3]*Royal Institute of Technology, STOCKHOLM, Sweden;* [4]*University Hospital, LINKPING, Sweden;* [5]*Sunderby Hospital, LULE, Sweden;* [6]*Karolinska Hospital, STOCKHOLM, Sweden;* [7]*Södersjukhuset, STOCKHOLM, Sweden;* [8]*St. Görans Hospital, Stockholm, Sweden*

Backgrounds. Anemia is a common complication of malignancy. Inflammatory cytokines reduce erythropoietin production and cause disturbances in iron metabolism, most notably impaired iron uptake and mobilization from iron stores. This situation, named functional iron deficiency, may be one reason why only ~60% of cancer patients respond to epoetin therapy. Previous studies reporting a potentiating effect of intravenous (IV) iron to epoetin therapy in cancer patients may not have excluded iron depletion as a cause of anemia. AIMS. To assess whether adjuvant IV iron therapy potentiates epoetin β (NeoRecormon™) treatment of anemia in patients with lymphoproliferative disorders (LPD) and proven iron presence in the bone marrow. *Methods.* NIFe (NeoRecormon with Intravenous Iron [Fe]), an open, prospective, randomized study in anemic patients with indolent LPD not receiving chemotherapy, was performed in 15 Swedish centers. Sixty-seven patients with indolent non-Hodgkin's lymphoma (n=19), chronic lymphocytic leukemia (n=23) or multiple myeloma (n=25) were randomized to receive either epoetin β only or both epoetin β and IV iron. Inclusion criteria were cancer-associated anemia (hemoglobin [Hb] ≥9 to ≤11 g/dL) and demonstration of stainable iron in a bone marrow aspirate. Major exclusion criteria were transfusion dependency, recent chemotherapy, serum ferritin >800 ng/mL or anemia from other causes. Epoetin β 30 000 IU once weekly (QW) was given subcutaneously for 16 consecutive weeks.



Dose adjustments were performed according to the label. Iron sucrose (Venofer™) 100 mg QW IV was given from week 0 to 6, followed by 100 mg every 2 weeks. The primary efficacy parameter was change in Hb level from baseline to end of treatment (EOT). Secondary endpoints were Hb response rates (% of patients with Hb 2 g/dL in the absence of red blood cell transfusion), dose of epoetin β and iron kinetics. All 67 randomized patients were included in the intention-to-treat (ITT) population, and 60 completed the study. Three patients received transfusion and/or chemotherapy and were not included in the per-protocol (PP) population of 57 patients. *Results.* There were no significant differences in key parameters between the two groups at baseline. The epoetin-plus-iron group had a significantly higher mean change in Hb level from baseline to EOT than the epoetin-only group (2.76 vs 1.56 g/dL [p=0.0002; ITT population] and 2.91 vs 1.50 g/dl [p<0.0001; PP population]). Hb response was reached earlier and in significantly more patients in both the ITT (79% vs 50%; p<0.02) and the PP (93% vs 53%; p=0.001) populations at EOT (Figure). Furthermore, a lower dose of epoetin was required in the group receiving iron compared with the group receiving epoetin alone (v=0.051). Conclusion: Compared with epoetin only, use of concomitant IV iron significantly increased Hb concentrations and the proportion of Hb responders in non-iron-depleted patients with LPD and cancer-associated anemia. Moreover, a lower dose of epoetin was needed to achieve these better and quicker hematopoietic responses.

0997

A NOVEL C TO G MUTATION IN THE HIF PROLYL HYDROXYLASE, PHD2, ASSOCIATED WITH ERYTHROCYTOSIS IN THREE FAMILY MEMBERS

M.J. Percy,[1] Q. Zhao,[2] A. Flores,[2] C. Harrison,[3] T.R.J. Lappin,[4] P.H. Maxwell,[5] M.F. McMullin,[4] F.S. Lee[2]

[1]*Belfast City Hosptial, Belfast, United Kingdom;* [2]*University of Pennsylvania, Phildelphia, Pennsylvania, USA;* [3]*St Thomas' Hospital, London, USA;* [4]*Queen's University, Belfast, United Kingdom;* [5]*Imperial College, London, United Kingdom*

Backgrounds. Red cell hemostasis is under the control of a highly sensitive negative feedback mechanism in which the glycoprotein hormone

erythropoietin (Epo) stimulates red cell production. Epo is synthesised by the kidney in response to hypoxia and the hypoxia-inducible factor (HIF) transcription complex, which consists of an α and a β subunit, regulates this process. Although both subunits are constitutively expressed, the α subunit is undetectable at the protein level due to continual targeting to the proteasome. In the presence of oxygen, members of the prolyl hydroxylase domain (PHD) group of enzymes actively hydroxylate prolines 402 and 564 in the oxygen dependent degradation (ODD) domain of HIF-1α. Upon hydroxylation the von Hippel Lindau (VHL) protein is able to associate and ubiquitinylation occurs, consequently the α subunit is proteasomally degraded. Although defects in the VHL gene have been identified in the many familial erythrocytosis cases, which are characterised by red cell hyperplasia and no identified secondary cause, there remains a considerable number of cases where the defect remains elusive. *Aims.* To screen members of the PHD family of prolyl hydroxylases for molecular defects individuals with erythrocytosis and assess the impact of any mutations detected on the Epo negative feedback pathway. *Methods.* DNA was prepared and PCR-direct sequencing of the three members of the PHD family was performed. *In vitro* binding and enzymatic functional assays were performed using *in vitro t*ranslated wild type and mutant PHD2 protein. *Results.* A heterozygous change of C to G at base 950 in PHD2 was detected in three erythrocytosis individuals from one family. All affected members exhibited subtly raised haematocrits with inappropriately normal Epo levels. The C950G base change was not detected in 200 normal control samples. This mutation results in loss of proline 317, located 2 amino acids away from an iron chelating residue in the active site, and replacement with arginine. The Pro317Arg mutant was found to exhibit reduced affinity for HIFα and its ability to hydroxylate HIFα was greatly impaired. *Summary*: *In vitro* binding and enzymatic assays have established that the Pro317Arg mutation would impair the function of PHD2, resulting in less HIF-1 α being hydroxylated and allowing more to escape proteasomal degradation. In addition, the mutation in PHD2 indicates that this is the main prolyl hydroxylase active in the regulation of HIF-1 α in the Epo pathway. There is now some evidence to suggest that deletion of PHD2 may play a role in the development of endometrial cancer thus raising the possibility that PHD2 may be analogous to VHL, where impaired function causes erythrocytosis while loss of function results in a cancer syndrome.

0998

SELECTED IMIDS IMMUNOMODULATORY DRUGS: NEW APPROACHES TO THE REGULATION OF ERYTHROPOIESIS AND HEMOGLOBIN SYNTHESIS IN HEMOGLOBINOPATHIES

A. Moutouh de Parseval,[1] D. Verhelle,[1] H. Brady,[1] E. Glezer[1], N. Richard,[1] U. Jhaveri,[1] K. Jensen-Pergakes,[1] L. Corral[1], C.L. Morris,[2] G. Muller,[1] K. Chan[1]

[1]*Celgene, San Diego, USA;* [2]*Loma Linda University School of Medicine, Loma Linda, USA*

Sickle cell anemia (SCA) and β-thalassemia constitute a public health problem worldwide and new therapies are needed. Inhibition of hemoglobin S (HbS) polymerization is a major target for therapeutic approaches in SCA. New experimental therapies including hydroxyurea (HU) have attempted to augment the synthesis of fetal hemoglobin (HbF) and improve upon current treatment. Clinical trial results have demonstrated that lenalidomide (Revlimid™), recently approved by the FDA, reduces or even eliminates the need for red blood cell transfusions in some anemic myelodysplastic patients. We have examined whether CC-4047 and lenalidomide, two distinct IMiDs (immunomodulatory drugs), currently under evaluation for the treatment of hematological cancers could regulate erythropoiesis and hemoglobin synthesis. For this purpose, we used an *in vitro* culture model to differentiate human erythroid progenitors from bone marrow or peripheral blood $CD34^+$ cells. We demonstrate that CC-4047 is a potent inducer of fetal hemoglobin (HbF) and synergize with hydroxyurea (HU) during erythroid differentiation of CD34 progenitors isolated from healthy and SCA donors. In addition CC-4047 and lenalidomide modulated erythropoiesis, slowing erythroid maturation and increased proliferation of immature erythroid cells. Unlike other inducers of fetal hemoglobin such as HU, 5-aza-cytidine and butyrate, CC-4047 and lenalidomide were not cytotoxic. Gene expression profiling of erythroid differentiated cells showed that our drug regulate specific erythroid transcription factors and genes that participate in hemoglobin synthesis, and genes involved in cell cycle and cellular differentiation. CC-4047 controls globin gene expression during erythroid differentiation by inducing sustained expression of fetal and embryonic hemoglobin synthesis. Our results support the hypothesis that CC-4047, alone or in combination with current approved therapies, can restore effective erythropoiesis and increase the ratio of fetal to adult hemoglobin. In addition, CC-4047 has the ability to inhibit TNF-α production and help to limit the inflammatory state in sickle cell patients. In conclusion, CC-4047 may represent an innovative new therapy for β-hemoglobinopathies.

0999

RESPONSE OF MYOCARDIAL T2* TO ORAL DEFERASIROX MONOTHERAPY FOR 1 YEAR IN 29 PATIENTS WITH TRANSFUSION-DEPENDENT ANAEMIAS; A SUBGROUP ANALYSIS

P. Eleftheriou,[1] M. Tanner,[2] D. Pennel,[2] J.B. Porter[1]

[1]*University College Hospital, London, United Kingdom;* [2]*Royal Brompton Hospital, London, United Kingdom*

Background and Aims. Patients with transfusion-dependent anaemias and iron overload who were entered into multicentre studies on deferasirox (Exjade) at University College London Hospitals (UCLH) had myocardial T2* performed at the Royal Brompton Hospital, as part of their routine monitoring. We have previously shown in 22 patients (16 β-thalassemia, 6 other chronic anemias) that treatment with deferasirox for 1 year at doses between 10 and 30 mg/kg/day is associated with an mean improvement in myocardial T2* of 6.4 ±1.76 ms, p=0.0026 (5.1ms geometric mean). (*Porter et al. , Blood 11, 3600, 2005*) *Patients and Methods.* We now report a total of 29 patients who had myocardial T2* assessment before and after 1year of treatment with deferasirox in studies 107 (randomised DFO vs deferasirox in β-thalassemia) and 108 (deferasirox monotherapy in β-thalassemia or other anemias). This is possible because an additional 7 patients, initially randomised to DFO on study 107, have now received deferasirox for 1 year. With larger patients numbers, sub-analysis of trends in myocardial T2* response has been undertaken; in particular we have examined whether improvement in myocardial T2* is similar in patients with baseline myocardial T2* above or below the current reference normal range of 20ms. All means are reported as geometric means as the relationship between T2* and tissue iron has been considered to be logarithmic. *Results.* The geometric mean myocardial T2* in the 29 patients improved from 18.7ms (25%-75% CI, 11.7-29ms) at baseline to 23.0ms (CI, 13.5-37) (p=0.0005) after 1 year of treatment. If patients are divided into those with normal myocardial T2* values (T2*>20ms) and those with shortening of myocardial T2* (T2*<20ms) before deferasirox treatment, a significant improvement in both subgroups was observed. In the group of 15 patients who had normal myocardial values before treatment, the mean T2* improved from 30.3ms (CI, 25.5-36.3ms) to 36.9ms (CI, 31.5-44.8) within a year (p=0.006). In the group of 14 patients, who had abnormal myocardial T2* before treatment, a mean T2* improved from 11.2ms (CI, 9.6-12.4) to 13.9ms (CI, 10.11-16.3) (p=0.019) after one year of treatment with deferasirox. After 2 years of treatment with deferasirox only 15 patients are as yet available for baseline, 1-year and 2-year follow up analysis. In these patients, the mean myocardial T2* improved from 16.1ms (CI, 11.9-25.6) to 22.5ms (CI, 15.3-29.5) after 2 years treatment with deferasirox (p=0.005). *Conclusion*: Deferasirox is associated with an improvement in myocardial T2* after 1 year of treatment with deferasirox, in patients both with decreased baseline and normal baseline T2* values. Patients treated with deferasirox for 2 years maintained the improvement of the myocardial T2*. Prospective multicentre trials are now planned to study the effects of deferasirox on myocardial T2* further.

Non-Hodgkin's lymphoma
Chronic lymphocytic leukemia - Experimental

1000

NOTCH-MEDIATED ACTIVATION OF PI-3K IS NEEDED FOR LYMPHOMAGENESIS IN T CELL-SPECIFIC PTEN-/- MICE

T.J. Hagenbeek,[1] M. Naspetti,[2] H. Spits[3]

[1]*Academic Medical Center, Amsterdam, Netherlands;* [2]*Netherlands Cancer Institute, Amsterdam, Netherlands;* [3]*Genentech, San Francisco, USA*

In the early stages of murine T cell development, cells go through two waves of proliferation: one mediated by IL-7 and stem cell factor, the other by triggering of the pre-TCR. An important downstream factor that is activated by IL-7 and the pre-TCR to positively regulate survival and proliferation is phosphatidylinositol-3 kinase (PI-3K). Active PI-3K phosphorylates phosphatidylinositol-(4,5)-biphosphate (PIP2) into phosphatidylinositol-(3,4,5)-triphosphate (PIP3), and this action is directly counteracted by Pten. Pten dephosphorylates PIP3 into PIP2. Previously we have shown that in mice before the age of 5 to 6 weeks, T cell-specific loss of Pten allows α-β lineage thymocytes to bypass IL-7 and pre-TCR mediated signaling, demonstrating a critical role of Pten in regulation of survival and growth of developing thymocytes. After 5 to 6 weeks of age T cell-specific $Pten^{-/-}$ mice showed the first clinical signs of T cell lymphomagenesis; all mice died within 25 weeks. Incubation of freshly isolated thymocytes or of established thymocyte cell lines from these mice with PI-3K inhibitors wortmannin or LY294002 induced a block in proliferation, and induced apoptosis. These data indicated that loss of Pten alone is not sufficient to drive survival and proliferation; activated PI-3K is still needed. To investigate which factors are involved in PI-3K activation, we crossed T cell-specific $Pten^{-/-}$ mice with mice that lacked IL-7 (γ $common^{-/-}$) and/or pre-TCR ($CD3\gamma^{-/-}$ or $RAG2^{-/-}$) signaling. All resulting double and triple knockout mice developed lymphomas. Active PI-3K was still needed to ensure survival and growth, since thymocytes from these mice showed a block in proliferation and induction of apoptosis after incubation with wortmannin or LY294002. Similar results were obtained when cells were treated with the γ secretase inhibitor IX (DAPT), which specifically blocks the Notch signaling pathway. Deltalike1-Notch signaling has been shown to phosphorylate Akt, a major downstream target of PI-3K, and *Notch1* has also been shown to be involved T cell lymphomagenesis in mice. These data indicate that Notch signaling greatly contributes to PI-3K activation in $Pten^{-/-}$ lymphoma cells *in vivo* to ensure survival and proliferation.

1001

ALLELIC SILENCING AT 13Q14.3: A NOVEL ONCOGENIC MECHANISM

D.M. Mertens,[1] S. Wolf,[1] C. Mund,[1] D. Kienle,[2] S. Ohl[1],
P. Schroeter,[1] F. Lyko,[1] H. Döhner,[1] S. Stilgenbauer,[1] P. Lichter[1]

[1]*DKFZ, Heildelberg, Germany;* [2]*Universitätsklinikum, ULM, Germany*

Background . Genomic material from chromosomal band 13q14.3 distal to RB1 is recurrently lost in a variety of human neoplasms. Lack of point mutations in candidate tumor suppressor genes and downregulation of these genes in tumors indicate an epigenetic pathomechanism localized in the critical region. *Aims*. Characterization of the epigenetic tumor suppressor mechanism localized in 13q14.3. *Methods*. Candidate tumor suppressor genes are down regulated by more than a factor of two in tumors with loss of one copy of the critical region. In addition, the presence of large non-coding RNA genes in 13q14.3 is reminiscent of imprinted regions where only one gene copy is active. Therefore we tested candidate tumor suppressor genes for monoallelic expression in healthy probands using single nucleotide polymorphisms and sequencing of RT-PCR products. Genotyping parents of these probands allowed allocation of the parental origin of either gene copy. In addition, we performed FISH experiments to measure replication timing of the two copies of the critical region to find out whether they are functionally different. As transcriptional activity and replication timing are effectuated by chromatin packaging, we used combined bisulfite-restriction (COBRA) analyses and bisulfite sequencing to assess DNA methylation of the critical region. Treatment of cultured cells with inhibitors of DNA-methyltransferases and histone-deacetylases allowed functional correlation of chromatin modification with expression of candidate tumor suppressor genes localized in the critical region. *Results.* In line with an imprinting mechanism, we find that the two copies of the critical region replicate asynchronously, suggesting differential chromatin packaging of the two copies of 13q14.3. In addition, we could detect monoallelic silencing of genes localized in the critical region and expression of one gene copy only. However, expression originated from either the maternal or paternal copy, excluding an imprinting mechanism. DNA methylation analyses showed one of the CpG islands of the region to be methylated. Demethylation of DNA and histone hyperacetylation induced biallelic expression, while replication timing was not affected. *Conclusions.* We propose that differential replication timing represents an early epigenetic mark that distinguishes the two copies of 13q14.3, resulting in differential chromatin packaging and monoallelic expression. This has profound effects for the tumor suppressor mechanism localized in 13q14.3: Deletion of the single active copy of the region at 13q14.3, which is lost in more than 50% of CLL tumors, or point mutations only in the active gene copies will suffice for complete loss of tumor suppressor function, as the remaining gene copies are epigenetically silenced. Thus, we provide a model for the pathomechanism of 13q14.3 in CLL by the interaction of genetic lesions and epigenetic silencing.

1002

DYNAMIC MODIFICATIONS OF THE SURFACE B-CELL RECEPTOR LIGHT CHAIN IN CASES OF HAIRY CELL LEUKAEMIA OCCURRING AT EXTRAFOLLICULAR SITES

F. Forconi,[1] T. Amato,[2] D. Raspadori,[1] S.S. Sahota,[3]
M.A. Dell'Aversano,[1] M. Tassi,[1] M. Defina,[1] D. Rossi,[4] L. Rigacci,[5]
F. Zaja,[4] F. Gherlinzoni,[4] S. Tavera,[4] L. Leoncini,[2] G. Gaidano,[4]
F.K. Stevenson,[3] F. Lauria[1]

[1]*Ematologia e Trapianti, Siena, Italy;* [2]*Anatomia Patologica, Siena, Italy;* [3]*Cancer Sciences Division, Southampton, United Kingdom;* [4]*Ematologia, Novara, Italy;* [5]*Ematologia Careggi, Florence, Italy*

Background. Ig gene analysis delineates critical features of the clonal history of a B-cell tumor. After antigen interaction, mature B-cells undergo somatic mutation of the V-genes and isotype switch events, generally in the germinal center (GC). Receptor revision by secondary recombination of the V-genes with re-expression of recombination activating gene (RAG) enzymes rarely occurs at this stage. From small series of cases, we have reported that most hairy cell leukemias (HCL) carry mutated VH-genes, with low levels of intraclonal heterogeneity, while a minor subset have unmutated VH-genes. Both subsets commonly have ongoing Ig isotype switch events and express activation-induced cytidine-deaminase (AID). However they lack CD27 and CD38 GC markers, and CD23, essential for lymph node entry. *Aims & methods*. In an expanded series of HCL (60 cases) with VH-genes available, the expressed VL (32) tumor-derived genes were evaluated to probe more fully the differentiation status of the cell of origin. *Results*. The majority (35/44, 79,5%) co-expressed multiple Ig isotype proteins on the HCs. From analysis of VH, VH3 family was most common (38/60, 63%), with significant preference of the VH3-30 and VH3-33 members (p<0,005). Most HCL (44/60) carried variable tiers of mutations in the VH-genes (77.13-97.95% homology to germline), with low level of intraclonal heterogeneity also documented in cases (13/60) with <2% deviation from germline, while 3/60 (5%) displayed completely unmutated VH-genes. Analysis of the light chains showed preferential use of surface λ chain (34/50, 62%), consistent with secondary rearrangement. VL-genes were evaluated in 16 κ and 16 λ expressing HCL. All (16/16) λ cases used Jλ3 segment. Thirty of 32 cases carried mutated VL-genes (94,75%-99.6%) with low levels of intraclonal heterogeneity, while 2 cases carried completely unmutated VL-genes, reflecting heterogeneity in mutational status as for the VH-gene. Strikingly, cloning of the tumor VL revealed inframe functional secondary rearrangements in 2/13 cases (Vk1 & Vk2 in Case R1, Vlambda1 & Vlambda2 in R2), most likely occurring in different tumor cells. Primary and secondary rearrangements showed mutations (98.1 and 99.6% homology in R1; 97.6 and 99.6% homology in R2). In both cases, RAG1 re-induction was also identified by RT-PCR and sequence verification. Both cases expressed AID transcripts, displayed intraclonal mutational variation in the VH and/or the VL-genes and 1/2 cases had ongoing isotype switch events. These data suggest a dynamic, on-going modification of the B-cell receptor (BCR) in HCs, including receptor revision, which occurs most likely in response to antigenic stimuli. N-glycosylation sites, commonly introduced by somatic mutation in the BCR of tumors of the GC, were not observed in the functional VH or VL-genes, to support the concept that tumor events occur outside the GC. *Conclusions*. These data confirm heterogeneity in the cell of origin in terms of mutational status, with a minor subset with unmutated V-genes. Restricted V-gene segment usage, and low levels of ongoing mutations with AID activated, coupled with the new observation of receptor revision and re-expression of RAG enzymes indicate that selective

BCR stimulation could be a promoting factor in HCL development at extrafollicular sites.

1003

REDIRECTION OF CMV SPECIFIC CTL TOWARDS B-CLL VIA CD20 TARGETED HLA/CMV COMPLEXES

R. Mous,[1] P. Savage,[2] E.B.M Remmerswaal,[3] R.A.W. van Lier[1], E. Eldering,[1] M.H.J. van Oers[1]

[1]*AMC, Amsterdam, Netherlands;* [2]*Charing Cross Hospital, London, United Kingdom;* [3]*Academic Medical Center, Amsterdam, Netherlands*

Backgrounds. Because B cell chronic lymphocytic leukemia (B-CLL) can not be cured with current therapies, but in general has a slow progression and rather long median survival, it is considered an attractive candidate for active T cell mediated immunotherapy. However B-CLL cells have poor antigen presenting capacity because they express low levels of co-stimulatory molecules. Moreover, most immunotherapeutic strategies require knowledge of the eliciting tumor antigen and/or ex vivo manipulation of patient cells. To circumvent these drawbacks we aim to redirect existing viral immunity towards B-CLL. Previously, we have shown that in patients with B-CLL considerably expanded numbers of cytomegalovirus (CMV)-specific CD45RAvCD27 $CD8^+$ cytotoxic T cells are present (*W. Mackus et al. Blood 2003; 102:1057*). These cells are potent cytotoxic effector cells when directed against B-CLL cells loaded with CMV peptide (*A Kater et al. Br.J. Haematol. 2004; 126:512*). *Aim:* To test a novel bridging reagent to redirect CMV-specific CTL to specifically target B-CLL. This targeting construct is composed of a streptavidin fused anti-CD20 single chain variable fragment (scFv) in combination with biotinylated MHC class I molecules containing CMV pp65 peptide (HLA/CMV). *Methods.* We evaluated CD20-HLA/CMV induced lysis of B-CLL cells by CMV specific CTL in a standard chromium release assay. Furthermore, we tested CD20-HLA/CMV induced proliferation of CMV specific CTL by CFSE staining and the induction of cytokine production via intracellular staining. *Results.* We demonstrate that the targeting complex is stable on the cell surface for ≥24 hours, and that B-CLL cells coated with this CD20-HLA/CMV complex can be lysed by autologous CMV-specific $CD8^+$ CTL with similar efficiency as B-CLL cells directly loaded with CMV-peptide. Killing occurs at scFv CD20 concentrations of ≥100 ng/ml and HLA/CMV concentrations of ≥20 ng/mL. HLA-A2 positive B-CLL cells coated with HLA-B7/CMV complexes were only lysed by HLA-B7 positive CMV-specific CTL, whereas HLA-A2/CMV complex targeted HLA-A2 positive B-CLL cells were unaffected by HLA-B7 positive CMV specific CTL, proving HLA restriction of the killing. Furthermore, CD20-HLA/CMV complex coated B-CLL cells induce both proliferation and cytokine production (interferon γ, tumor necrosis factor α, and macrophage inflammatory protein-1β) in CMV-specific $CD8^+$ T cells. Finally, we explored the requirements for interferon γ production by CMV specific T cells using blocking antibodies against LFA1, LFA2, CD80 and CD45. We demonstrate that immunological synapse formation around CD20-HLA/CMV is different from the synapse formation around pp65 loaded autologous MHC-I. Notably, lysis of both CLL cells coated with CD20-HLA/CMV complex or directly loaded with CMV-peptide could only be blocked by anti-LFA1 antibodies (and not by LFA2 or CD80 blocking antibodies). This indicates that lytic synapse formation requires only a limited number of molecules. *Summary/Conclusions.* CD20-HLA/CMV complexes elicit both immune activation and direct cytotoxicity towards B-CLL cells. The findings of our study constitute a necessary step towards possible application of CD20-HLA/CMV complexes for immunotherapy of B cell malignancies. It is obvious that this recently recognized capacity to redirect existing antiviral immunity towards tumor cells has a utility in cancer immunotherapy far beyond CMV and B-CLL.

1004

THE CYCLIN-DEPENDENT KINASE INHIBITOR SELICICLIB ENGAGES THE MITOCHONDRIAL APOPTOSIS PATHWAY VIA THE MCL-1/NOXA AXIS IN B-CLL

Y.H. Hallaert,[1] R. Spijker,[1] M. Jak,[1] A. Jaspers,[1] I.A.M. Derks[1], N.L. Alves,[1] J.P. de Boer,[2] D. de Jong,[2] S.R. Green,[3] R.A.W. van Lier,[1] M.H.J. van Oers,[1] E. Eldering[1]

[1]*AMC, Amsterdam, Netherlands;* [2]*NKI, Amsterdam, Netherlands;* [3]*Cyclacel Ltd., Dundee, United Kingdom*

Backgrounds. Seliciclib (R-roscovitine) is a cyclin-dependent kinase inhibitor in clinical development. It triggers apoptosis by inhibiting *de novo* transcription of the short-lived Mcl-1 protein. It is not known how Mcl-1 degradation triggers Bax or Bak activation that is required for most forms of cell death. *Aims.* We studied the effects of seliciclib on apoptosis genes and Mcl-1 protein interactions in B cell chronic lymphocytic leukemia (B-CLL), a malignancy with known aberrant apoptosis regulation. *Methods.* The effect of seliciclib on viability and apoptosis gene expression pattern (RT-MLPA) was investigated. Purified B-CLL cells (PBMC consisting of >90% B-CLL cells) and Ramos cell lines overexpressing different apoptosis regulators were used in this study. Western blotting and immunoprecipitation assays were performed. Ramos cells were transduced with retroviral vectors expressing Noxa siRNA or control-GFP virus. The effect of reduced Noxa levels was tested for different apoptosis stimuli. *Results.* Seliciclib induced apoptosis in B-CLL irrespective of IgVH mutation or p53 status, and without induction of the p53-responsive Bax and Puma transcripts. Analyses of B cell lines overexpressing distinct apoptosis regulators (Bcl-2, caspase-9DN, FLIP-S, FADD-DN) established that seliciclib triggered the mitochondrial apoptosis pathway. In B-CLL cells, pro-survival Mcl-1 was engaged by the pro-apoptotic proteins Noxa and Bim but not by Bak. The importance of Noxa as a mediator of seliciclib-induced apoptosis was demonstrated via RNAi in two model systems. Noxa knock-down resulted in inhibition of apoptosis after seliciclib, but not by fludarabine, staurosporin, CD95- or BCR-triggering. *Conclusion*: Since Noxa is elevated in B-CLL, its involvement in p53-independent apoptosis suggests this BH3-only protein may be a therapeutic target.

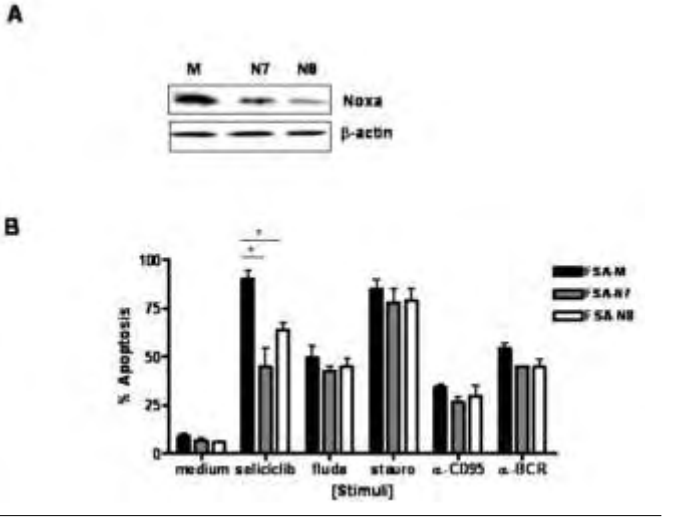


Figure 1. Noxa RNAi afford selective resistance to selicicl.

Pharmacogenetics and molecular targeting

1005

PHARMACOGENETIC ANALYSIS OF POLYMORPHISMS IN CYP3A4, CYP3A5, GSTP1, GSTM1, GSTT1 AND MDR1 GENES FOR SURVIVAL AND THERAPY RELATED TOXICITY IN MULTIPLE MYELOMA

A. Broyl,[1] C. Schilthuizen,[1] B. van der Holt,[2]
Y. de Knegt,[1] R.H.N. van Schaik,[1] R.A. Raymakers,[3] H.M. Lokhorst,[4] E. Vellenga,[5] E. Kamst,[1] P. Sonneveld[1]

[1]*Erasmusmc, ROTTERDAM, Netherlands;* [2]*ErasmusMC-Daniel den Hoed Cancer Center, ROTTERDAM, Netherlands,* [3]*Radboud University Medical Center, NIJMEGEN, Netherlands;* [4]*University Medical Center Utrecht, UTRECHT, Netherlands;* [5]*University Medical Center Groningen, GRONINGEN, Netherlands*

Background. Cytochromes *P450* (CYP450) and gluthatione-S-transferases (GSTs) are drug metabolizing enzymes involved in detoxification of chemotherapeutic agents. Genetic polymorphisms in these genes are frequent and may alter the metabolism of anti-cancer drugs. Among these, the CYP3A4*1B (290A→G) polymorphism affects the promoter region. Polymorphisms in the CYP3A5 (6986A→The 3435C→T polymorphism in the MDR1 gene alters protein expression. Homozygous deletions in the *GSTM1* and *GSTT1* genes result in absence of the enzyme. We hypothesized that inherited mutations in these enzymes may result in different treatment response and toxicity in multiple myeloma. Aims. We investigated the relevance of different genetic polymorphisms of these enzymes for clinical outcome in multiple myeloma patients treated in the HOVON 24 prospective clinical phase III study, a clinical trial comparing single with double intensive therapy. *Methods.* Genetic polymorphisms were determined in DNA isolated from peripheral blood of 211 myeloma patients treated in the *HOVON 24*. Polymorphisms in different genes were detected using a restriction fragment length polymorphism (RFLP)-PCR and frequencies were analyzed with outcome. *Results.* For the *CYP3A4*, *GSTT1*, *GSTM1* and *MDR1* genes no significant influence was observed for partial remission (PR), complete remission (CR), event free survival (EFS), progression-free survival (PFS), time to progression (TTP), overall survival (OS) and toxicity. However, patients with two variant alleles for the GSTP1 gene had significantly lower remission rates (p=0.01). Patients with the homozygous genotype of the *CYP3A5* mutant allele showed improved PFS (p=0.04) and OS (p=0.01) and an increase of TTP (p=0.03). Summary/ conclusions. This is the first study that shows a significant association between the CYP3A5*3 polymorphism and outcome for a cohort of MM patients treated in a clinical trial comparing single with double intensive therapy. Patients with two variant alleles for the GSTP1 gene had significantly lower remission rates, although this did not translate into significant differences in EFS, PFS, TTP and OS. Currently the results of this biological study are evaluated using a multivariate comparison of these data with the clinical prognostic factors. Secondly, these results will be validated in a cohort of 400 patients treated in a subsequent HOVON 50 trial. The results will be presented in a comprehensive analysis of both clinical and pharmacogenetic variables.

1006

EXPOSURE OF LEUKEMIC CELLS TO ANTHRACYCLINES INDUCES RAPID AND BROAD UPREGULATION OF ATP BINDING CASSETTE TRANSPORTERS

P.L.M. de Grouw,[1] A. Buda,[2] A. van der Reijden,[1] J. de Witte[1],
H. Jansen,[1] A.P. Raymakers,[1] H.G.P. Raaijmakers[1]

[1]*Radboud University Medical Centre, NIJMEGEN, Netherlands;* [2]*University of Pisa, PISA, Italy*

Drug efflux by ATP-binding cassette (ABC) transporters is a well-established mechanism by which leukemic cells evade chemotherapeutical eradication. The role of most ABC transporters in chemoresistance of leukemic cells, however, remains unknown. The dynamics of ABC transporter expression in leukemic cells upon exposure to chemotherapeutical agents could identify novel transporters involved in drug resistance. We profiled gene expression of all 45 transmembrane ABC transporters after short-term drug exposure with anthracyclins (mitoxantrone and daunorubicin) in leukemic progenitor cells (KG1a cell line and primary AML CD34+ cells) by real-time RT-PCR on micro fluidic cards. In KG1a cells significant induction (> 2-fold) of many ABC transporters was observed within 72 hours of exposure to both mitoxantrone and daunorubicin (23 and 33 transporters respectively). Of these induced transporters, 12 transporters show an upregulation of more than 20-fold up to 850-fold for ABCA4 after exposure to mitoxantrone, and after exposure to daunorubicin the induction increases up to 2800-fold for ABCA6. Among the top 12 of highest induced genes, 8 transporters were overlapping between mitoxantrone and daunorubicin treated cells, including three known drug resistance genes (ABCG2, ABCC6 and ABCB11). The remaining highest up regulated genes are currently not associated with drug resistance. Rapid and broad induction of ABC transporters upon exposure to anthracyclins was confirmed in primary CD34+ leukemic cells *in vitro* (n=2 patients). In the first patient sample 24 and 13 ABC transporters were more than two-fold up regulated after mitoxantrone and daunorubicin exposure respectively. In the second patient sample 11 and 15 ABC transporters were up regulated after exposure to mitoxantrone and daunorubicin respectively. There was a large overlap between the induced transporters after exposure to mitoxantrone and daunorubicin between patient samples and within patient samples. In the top ten of most induced genes were 7 transporters known to be involved in drug resistance, including the above mentioned 3 drug transporters induced in the KG1a cell line. Also in the patient samples the remaining transporters are currently not associated with drug resistance. These data show that short-term drug exposure rapidly induces a large range of ABC transporters in leukemic progenitor cells. These included both known drug transporters and transporters not previously associated with drug resistance. The findings challenge the rationale of inhibition of single transporters to circumvent drug resistance of leukemic progenitors and warrant further research into the role of novel ABC transporters in chemoresistance of leukemic cells.

1007

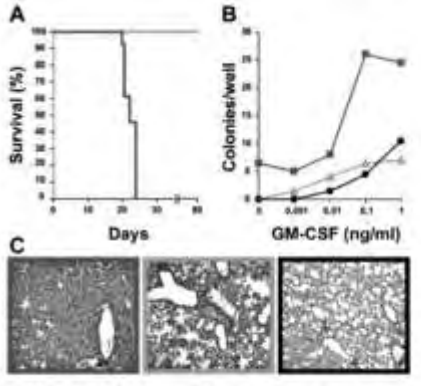
TARGETED INACTIVATION OF GERANYLGERANYLTRANSFERASE TYPE I RESCUES MICE FROM LETHALITY INDUCED BY ONCOGENIC K-RAS EXPRESSION IN GRANULOCYTES AND MONOCYTES

O. Bergo,[1] A.K. Sjogren,[1] K.A. Andersson,[1] A.S. Svensson[1],
M.L. Liu,[1] B.C. Cutts,[1] C.K. Karlsson,[1] M.D. Dalin,[1] S.G. Young[2]

[1]*Institute of Medicine, Goteborg, Sweden;* [2]*UCLA, Los Angeles, USA*

Backgrounds. Ras proteins are isoprenylated at a carboxyl-terminal CAAX motif by farnesyltransferase (FTase) and geranylgeranyltransferase type I (GGTase-I), respectively. Activating mutations in Ras genes are very common in human cancers, including hematological malignancies. Thus, the Ras proteins are attractive anticancer drug targets. One strategy to block Ras signaling is to inhibit the enzymes that modify the CAAX motif. Inhibitors of FTase (FTIs) have shown efficacy in the treatment of some cancers. However, both K-Ras and N-Ras can be isoprenylated by GGTase-I in the setting of FTI therapy. Consequently, we are also focusing on GGTase-I. Several inhibitors of GGTase-I (GGTIs) have been synthesized: whereas all GGTI compounds inhibit GGTase-I activity, some cause growth arrest and others induce apoptosis and are lethal in mice. These compound-specific differences among different GGTIs have made it hard to understand their mechanism of action. Therefore, a thorough understanding of the impact of GGTase-I deficiency is warranted. *Aims.* 1. To define the role of GGTase-I in cell viability and proliferation; 2. To develop an in vivo model of K-Ras-induced leukemia; and 3. To test the hypothesis that inhibition of GGTase-I would block the development of K-Ras-induced leukemia. *Methods.* We have generated mice with a Cre-inducible GGTase-I knockout allele (Pggt1bflx). Moreover, we developed a new mouse model of leukemia, based on Cre-loxP techniques, where the expression of oncogenic K-Ras was targeted to granulocytes and monocytes. For this, we used mice with an oncogenic mutation (G12D) in the Kras2 locus (Kras2LSL). In the absence of Cre, this K-Ras allele is silent. Induction of Cre turns on the expression of K-RasG12D. We have bred mice with the Kras2LSL allele and a Cre transgene driven by the lysozyme M promoter (Lysm-Cre): In those mice, Cre expression, and subsequently K-RasG12D expression, is targeted to granulocytes and monocytes. Finally, to determine if inhibition of GGTase-I would block K-Ras-induced transformation in vivo, we used Cre to simultaneously activate the expression of K-RasG12D and inactivate the expression of Pggt1b in the same cells. In this way, we determined if the absence of Pggt1b would prevent the development of K-Ras-induced leukemia. *Results.* We found that the Cre-induced knockout of Pggt1b abolished GGTase-I activity and was compatible with cell viability in both fibroblasts and myeloid cells and caused proliferating fibroblasts to enter cell cycle arrest. Next, we developed a new mouse model of leukemia, where K-RasG12D expression was targeted to granulocytes and monocytes. We found that the Kras2LSL/+Lysm-Cre mice developed leukocytosis, splenomegaly, infiltration of cells in the liver,

and a massive recruitment of inflammatory cells in the lungs. The mice had to be sacrificed three weeks after birth. Finally, the knockout of Pggt1b rescued mice from this lethal K-Ras-induced leukemia (Figure), reduced spleen size, and the infiltration of cells in the lungs. *Conclusions.* Cells lacking GGTase-I enzymatic activity are viable but are unable to proliferate and the knockout of Pggt1b rescues mice from a lethal K-Ras-induced leukemia. This mouse model and genetic strategies should be valuable tools to study mechanisms and treatment of Ras-induced hematological malignancies.



A knockout of GGTase-I rescues mice from a lethal K-Ras-induced leukemia.
A. Kaplan-Meier curve showing the rapid demise of *Kras2*$^{LSL/+}$Lysm-*Cre* and *Pggt1b*$^{flx/+}$*Kras2*$^{LSL/+}$Lysm-*Cre* (expressing oncogenic K-Ras in granulocytes and monocytes and 100 and 50% of GGTase-I activity, respectively; RED, $n = 15$); and the survival of *Pggt1b*$^{flx/flx}$*Kras2*$^{LSL/+}$Lysm-*Cre* mice that lack GGTase-I activity in the same cells (GREEN; $n = 8$; $P < 0.0001$).
B. Preliminary characterization showing an increased sensitivity to GM-CSF of splenocytes from *Pggt1b*$^{flx/+}$*Kras2*$^{LSL/+}$Lysm-*Cre* and *Kras2*$^{LSL/+}$Lysm-*Cre* mice (RED; $n = 1$) compared to splenocytes from "rescued" (GREEN) and wild-type (BLACK) mice.
C. Lung tissue showing massive proliferation of cells in the lungs of *Kras2*$^{LSL/+}$Lysm-*Cre* mice (RED frame); lungs from "rescued" *Pggt1b*$^{flx/flx}$*Kras2*$^{LSL/+}$Lysm-*Cre* mice showing a partial phenotype (GREEN frame); and normal lung tissue for comparison (BLACK frame)

1008

FLT3 MUTATED PEDIATRIC ACUTE MYELOID LEUKEMIA (AML) SAMPLES ARE SENSITIVE TO THE TYROSINE KINASE INHIBITOR SU11657

B.F. Goemans,[1] C.H. Zwaan,[2] D. de Lange,[3] J. Cloos,[3] Q. Waisfisz,[3] D. Reinhardt,[4] K. Hahlen,[2] B.E. Gibson,[5] U. Creutzig,[4] G.J.L Kaspers[3]

[1]*VU university medical center, Amsterdam, Netherlands;* [2]*Sophia Children's Hospital, Erasmus MC, Rotterdam, Netherlands;* [3]*VU University Medical Center, Amsterdam, Netherlands;* [4]*AML BFM Study Group, Hannover/Münster, Germany;* [5]*UK Childhood Leukaemia Working Party, Glascow, United Kingdom*

Despite intensive treatment regimens only 60% of pediatric AML patients survive. Therefore novel treatment strategies strategies to improve the outcome of pediatric AML are required. Almost 20% of pediatric AML patients harbor a FLT3 mutation (12% FLT3/ITD and 7% FLT3 D835 point mutations). Patients with a FLT3/ITD mutation have a poor prognosis. Tyrosine kinase inhibitors are novel drugs specifically targeting activated tyrosine kinases. SU11657 is one of these novel drugs and is a selective inhibitor of the tyrosine kinase receptors FLT3, KIT, PDGF and VEGF-R2. SU11657 is comparable to the currently approved SU11248 (sunitinib malate, Sutent®). In a phase I trial of sunitinib malate in AML all patients with FLT3 mutations (n = 4) had complete or partial morphologic responses compared with 2 of 10 evaluable patients with wild-type *FLT3*. These responses were of short duration, although longer in patients with mutated than wild type FLT3. In this study we investigated whether primary pediatric AML samples were sensitive to SU11657 *in vitro* and whether the effects of SU11657 are related to mutations in *FLT3* and KIT. We studied 70 pediatric AML samples for *FLT3/ITD* mutations using genescan analysis and *FLT3 D835* using light-cycler analysis. The KIT mutational analysis is in progress. These 70 pediatric AML samples were also tested for *in vitro* sensitivity to SU11657 using the 4 day total cell kill MTT assay (concentration range 0.0098 - 10 µM). Two measures of sensitivity were calculated: 1. The LC50 value (the concentration at which 50% of the cells is killed); 2. The percentage of cells surviving at 0.625 µM SU11657 (the concentration which discriminates best between sensitive and resistant samples). A FLT3/ITD mutation was detected in 20 samples (29%) and a FLT3 D835 mutation in 4 samples (6%). There was a 1000 fold difference in LC50 value between the most sensitive and the most resistant sample. Dose-response curves differed from what is normally observed in the MTT assay, as even high concentrations did not result in impressive cell-kill, as was also observed by others for FLT3 inhibitors when testing primary samples. AML samples without FLT3 mutations were resistant to SU11657 (median LC50=7.4 µM, median cells survival at 0.625 µM SU11657=91%). In contrast FLT3/ITD positive samples were significantly more sensitive to SU11657 [median LC50=3.9 µM (p=0.037), median cell survival at 0.625 µM SU11657= 66% (p<0.0001)]. The 4 FLT3 D835 mutated samples were sensitive to SU11657 [median LC50=2.4 µM (FLT3 negative vs. D835 p=0.16), median cell survival at 0.625 µM SU11657=64% (FLT3 negative vs. FLT3 D835 p=0.017)]. Despite these differences there was overlap in individual LC50 values, with some FLT3 non-mutated samples being relatively sensitive, and some FLT3mutated samples being relatively resistant to SU11657. We are currently performing KIT mutational analysis which may explain this sensitivity in non-FLT3 mutated samples. In conclusion, there is large interpatient variation in sensitivity to SU11657. Both FLT3/ITD and FLT3 D835 positive pediatric AML samples were more sensitive to SU11657 *in vitro* than FLT3 negative samples.

1009

DEVELOPMENT OF AN EFFECTIVE SAFETY SWITCH FOR SELECTIVE ELIMINATION OF HUMAN T CELLS IN VIVO AFTER ADOPTIVE TRANSFER

T. van Meerten,[1] H. Rozemuller,[1] W. Mackus,[2] P. Parren,[2] J. van de Winkel,[2] M.-J. Claessen,[1] R.S. van Rijn,[1] A. Hagenbeek[1], A. Martens,[1] S. Ebeling[1]

[1]*University Medical Center Utrecht, Utrecht, Netherlands;* [2]*Genmab BV, Utrecht, Netherlands*

Backgrounds. Adoptive transfer of T cells is frequently associated with unwanted side effects. In order to tackle these effects one could introduce a safety switch into the cells that permits their selective in vivo elimination. The human CD20 gene in combination with CD20 antibodies was recently proposed as a novel safety switch. In such a system, T cells may be genetically modified with a CD20-encoding vector prior to adoptive transfer. If necessary, CD20-transgenic cells can be eliminated in vivo through administration of CD20 antibodies, such as the chimeric antibody rituximab (RTX) that is currently used to treat CD20$^+$ lymphoma cells. RTX activates the complement system and recruits immune effector cells, resulting in rapid death of CD20$^+$ cells. Recently, a novel human CD20 antibody, Humab 7D8, was shown to have superior activity over RTX. *Aims and Methods.* In this study a set of CD20-encoding retroviral vectors was generated, which either lacked or contained one or both of two regulatory elements: 1) the woodchuck post-transcriptional regulatory element (WPRE) to increase CD20 expression, and 2) the chicken hypersensitivity site 4 insulator element (INS) to achieve a position independent expression of CD20 and to increase the safety profile of the vector by preventing activation of cellular (onco)genes by the retroviral enhancer. *Results.* We found that the level of CD20 expression obtained with vectors containing INS was 2-fold lower than with vectors lacking INS. Additional inclusion of WPRE restored the level to that of the vector without INS. In addition, INS greatly enhanced the homogeneity of CD20 expression in T cells. Moreover, after 3 months in culture, all cells generated with CD20-INS had retained CD20 expression, while 60% of cells transduced with the control CD20 vector had lost CD20 expression. Complement dependent cell kill (CDC) of both RTX and HuMab 7D8 was dependent on the level of CD20 expression (p<0.01). However, while very low CD20-expressing cells were completely resistant against RTX they could be effectively killed by HuMab 7D8. For maximal kill of CD20-high cells, a 100-fold lower dose of HuMab 7D8 was required, compared to RTX. In vivo efficacy was studied through bioluminescent imaging of luciferase+ CD20-transgenic T cells. After transfer of CD20$^+$ cells in immune deficient RAG2-/-γc-/- mice, both CD20 antibodies were capable of eliminating >99% of CD20$^+$ cells, prolonging survival of mice from 20 till 42 days. Summary: We developed a safe vector that leads to homogeneous and stable expression of CD20 on human T cells. These cells can be killed effectively in vivo with HuMab 7D8, a recently developed CD20 antibody. This system will be applicable to other approaches that require inclusion of a safety switch in *ex vivo* modified cells.

Cancer genetics and cytogenetics in lymphoid diseases

1010

MOLECULAR KARYOTYPING BY HIGH RESOLUTION ARRAY CGH UNCOVERS AMPLIFICATIONS AND HOMOZYGOUS DELETIONS IN CD138 + SELECTED PRIMARY MULTIPLE MYELOMA CASES

C. Largo,[1] B. Saez,[2] J. Suela,[1] B. Ferreira,[1] S. Alvarez,[1] D. Blesa[1], F. Prosper,[3] M.J. Calasanz,[2] J.C. Cigudosa[1]

[1]*CNIO, Madrid, Spain;* [2]*University of Navarra, Pamplona, Spain;* [3]*University Clinic, University of Navarra, Pamplona, Spain*

Multiple Myeloma (MM) is a malignancy of clonal plasma cells with a wide variability in clinical features, responses to treatment, and survival times among patients. In 50% of the cases, the neoplastic transformation begins with a chromosomal translocation that juxtaposes the IGH gene locus to an oncogene. In addition, other genetic aberrations, as gains and/or losses of genomic regions (including trisomies and monosomies) are frequent but less characterized and they may contribute to the tumour phenotype Our objective was to characterize copy number changes present on CD 138 + multiple myeloma primary samples by means of DNA hybridization onto high resolution array CGH platform. We conducted a high resolution analysis of copy number changes on MAC´S sorted CD138 + myeloma cells. Twenty-six newly diagnosed MM samples at diagnosis were included in the study. 85% of the patients carried a normal karyotype at diagnosis. The median age of our patients was 67.5 years (range: 34-85). There were 16 men and 10 women in our series. The presence of IGH rearrangement has been analyzed using LSI IGH Dual Colour, Break Apart Probe (Vysis). For molecular karyotyping Human Genome CGH Microarray 44A /B platform from Agilent Technologies (Palo Alto, CA) was used. This platform contains 44.000 60-mer oligonucleotides covering the human genome with an average resolution of 45 Kb. CGH-Analytics 3.2.25 Agilent Technologies (Palo Alto, CA, USA) was used for the array analysis. RESULTS. Genomic copy number analysis, performed on selected cells, allowed the identification of a high number of deletions and gains. FISH screening revealed that 9 out 26 (35%) samples harboured an IgH rearrangement. We have discovered 267 copy number changes with a median of 8.5 changes per case, ranging from 2 to 26 copy number changes per case. By this CGH approach, we characterized whole chromosome 3, 5, 7, 9 and 15 gains in 50% of the samples. This defined the hyperdiploid group in ours series. Chromosome 13 deletions have been found in 38% of the cases. Gains of chromosome 19p, 1q gain, and a novel duplication of Xq21-qter were found to be among the most frequent aberrations. In addition to big structural changes, we have also identified small rearranged regions (below 500 Kb of size).Of great genetic relevance was the finding of homozygous deletions in chromosomes 6q, 11q, 13q and Xq, and the description of genomic amplifications in 6q, 9q, 16q and 17q. Finally we have established 68 common minimal rearranged regions that will be used in unsupervised and supervised clustering. This is the first time that high resolution array CGH analysis is carried out on CD 138+ on MM primary samples. This approach has allowed us to identify copy number changes in 100% of the samples and has made possible the identification of great genetic relevance homozygous deletions and amplifications in MM.

1011

MOLECULAR ANALYSIS OF PATIENTS WITH T-ALL USING ARRAY-CGH ENRICHED IN PROBES COVERING TYROSINE KINASE GENES

I. Lahortiga,[1] C. Graux,[2] K. van Roosbroeck,[1] N. Mentens[1], K. de Keersmaecker,[1] F. Lambert,[3] P. Vandenbergue,[1] I. Wlodarska[1], G. Froyen,[1] M.D. Odero,[4] P. Marynen,[1] J. Cools[1]

[1]*VIB, LEUVEN, Belgium;* [2]*Cliniques Universitaires St-Luc, Bruxelles, Belgium;* [3]*Pathology Institute, CHU Sart-Tilman, Liege, Belgium;* [4]*CIMA, Pamplona, Spain*

Background. Molecular analysis of T-cell acute lymphoblastic leukemia (T-ALL) has provided evidence for a stepwise alteration of thymocytes during transformation to leukemic T-cells. Genetic alterations in hematopoietic precursor cells lead to loss of cell cycle control, impaired differentiation, proliferation and survival advantages, and unlimited self-renewal capacity. These defects include inactivation of CDKN2A (P16) present in 96% of the patients, deregulated expression of transcription factors, and mutation of NOTCH1 present in 56% of patients. The molecular lesions leading to the proliferative and survival advantages of T-ALL cells are less well characterized and remain unknown in 80% of the T-ALLs. We have previously shown that cryptic deletions and amplifications can result in the generation of fusion genes. Examples of these are the cryptic 800 kb deletion on chromosome 4q12, and amplification of a 500 kb region of 9q34, resulting in the generation of *FIP1L1-PDGFRA* and NUP214-ABL1 fusion genes. *Aims*. Our aim was to set up a genome-wide analysis of T-ALL in order to detect cryptic deletions and amplifications, with a special focus on the 90 protein tyrosine kinase genes present in the human genome. *Methods*. We used the array-CGH (microarray comparative genomic hybridization) technology with slides containing genomic BAC probes spaced every 1 Mb over the human genome. An additional 480 probes were added covering the genomic locations of each of the 90 protein tyrosine kinases genes. *Results.* We performed array-CGH on 20 T-ALL cases. An interstitial deletion on chromosome 9p24 directly upstream of JAK2 was identified in 1 case. The deletion was confirmed by FISH. Quantitative PCR analyses indicated that the deletion was 700 kb in size including exons 1-3 of JAK2. Molecular analyses to characterize the possible presence of a fusion transcript involving JAK2 are in progress. No other rearrangements involving tyrosine kinase genes were observed in 19 other T-ALL cases, suggesting that cryptic deletions or amplifications involving tyrosine kinase genes are relatively rare in T-ALL. The most frequent aberration was the deletion of CDKN2A (14 cases). MYB duplication was found in two cases, and was confirmed by quantitative PCR. PTEN deletion was present in one case. Other unbalanced aberrations of various size were detected: del(6q) in 3/17 cases ranging from 5 to 33 Mb, del(9p) in 4/17 cases ranging from 4 to 43 Mb, dup(7q) in 2/17 cases and, dup(2q), del(7p), dup(9p) and del(12p) in one case each. Some of these rearrangements were not observed by standard cytogenetics. *Conclusions.* We detected a novel cryptic rearrangement of JAK2 in one T-ALL case, and duplication of MYB in two T-ALL cases. Molecular analysis of these cases, and array-CGH analysis of an additional 20 T-ALL cases and 10 T-ALL cell lines is ongoing.

1012

GENE EXPRESSION PROFILING OF APOPTOSIS GENES IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA

M.L. Den Boer,[1] A. Holleman,[1] R.X. Menezes,[1] M.H. Cheok,[2] G.E. Janka-Schaub,[3] W.E. Evans,[2] R. Pieters[1]

[1]*Erasmus MC-Sophia Children's Hospital, Rotterdam, Netherlands;* [2]*St. Jude Children's Research Hospital, Memphis, USA;* [3]*COALL study group, Hamburg, Germany*

The response to cytotoxic drugs varies amongst different subtypes of childhood acute lymphoblastic leukemia (ALL). We studied the expression of 70 apoptosis genes by Affymetrix U133A GeneChip microarrays in leukemic cells taken at initial diagnosis of 190 children with ALL. The expression of 44 out of these 70 genes differed significantly between T-lineage and B-lineage ALL, 22 genes differed in hyperdiploid versus non-hyperdiploid, 16 in TEL-AML1 positive versus negative, and 13 in *E2A*-rearranged versus negative B-lineage ALL. The data indicate that the expression of apoptosis genes highly differs between these leukemic subtypes. Expression of *MCL1* and *DAPK1* was significantly associated with prednisolone resistance, whereas *BCL2L13*, *HRK* and *TNF* were related to L-asparaginase resistance. Multivariate analysis including known risk factors revealed that *BCL2L13* expression was an independent prognostic factor (p=0.011). The same trend was observed in a validation group of 92 children with ALL treated on a different protocol at the St. Jude Children's Research Hospital (p=0.051). In conclusion, apoptosis genes are differentially expressed between subtypes of acute lymphoblastic leukemia. Out of 70 studied apoptosis genes, only 5 genes were associated with cellular drug resistance in childhood ALL. Functional studies addressing the causal relationship between these genes and drug resistance are currently being performed.

1013

CYCLIN D2 DYSREGULATION BY CHROMOSOMAL TRANSLOCATIONS TO TCR LOCI IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIAS

E. Clappier, W. Cuccuini, J.M. Cayuela, D. Vecchione, A. Baruchel, H. Dombret, F. Sigaux, J. Soulier

Hopital Saint-Louis, Paris France

D-type cyclins are key regulators of progression through G1 phase of the cell cycle. Strong expression of at least one of the three D cyclins is common in human cancers. However, while the cyclin D1 and D3 genes (*CCND1* and *CCND3*) are recurrently involved in genomic rearrangements, especially in mantle-cell lymphoma and multiple myeloma, no clear involvement of the cyclin D2 gene (*CCND2*) has been reported to date in human malignancies. In T-cell acute lymphoblastic leukemia (T-ALL), the T-cell receptor genes *TCRA/D* and *TCRB* are frequently involved in chromosomal rearrangements and deregulate oncogenes. In order to identify new chromosomal rearrangements and oncogenes in human T-ALL, we performed an interphasic FISH screening of T-ALL cases using TCR flanking probes. By this approach, we identified two new chromosomal translocations: t(7;12)(q34;p13) and t(12;14)(p13;q11), involving the *TCRB* and *TCRA/D* loci, respectively. Molecular analysis of the breakpoint derivative sequences demonstrated the involvement of the *CCND2* locus at 12p13. Expression analysis using RQ-PCR and immunoblotting demonstrated dramatic cyclin D2 overexpression in the translocated cases (n=3) compared to other T-ALLs (total, n=86), whereas other genes located near the translocation breakpoints were not deregulated on microarray analysis. To further evaluate expression in T-ALL with respect to normal T-cell differentiation, we analyzed *CCND2* expression in purified subpopulations from normal human thymus. *CCND2* levels were downregulated through progression from the early stages of normal human T-cell differentiation and transition to β-selection. In the most immature T-ALLs, a moderate *CCND2* expression was observed, consistent with their differentiation stage, while low expression was found in other T-ALL. By contrast, the massive and sustained expression in the CCND2-rearranged T-ALL cases strongly suggested oncogenic function due to the TCR translocation. T-ALL oncogenesis is a multi-step process. We here found that the *TCR-CCND2* translocations were associated with other oncogene expression (TAL1, *HOXA*, or *TLX3/HOX11L2*), *NOTCH1* activating mutations, and/or *CDKN2A/p16/ARF* deletion, showing that cyclin D2 dysregulation could contribute to multi-event oncogenesis in various T-ALL groups. In conclusion, this report is the first clear evidence of a direct involvement of cyclin D2 in human cancer due to recurrent somatic genetic alterations. This reinforces the view that the strong expression of cyclin D2 which is detected in various types of cancer including T-ALL cases can contribute to oncogenesis, and points to cyclin D2 as a potential target for therapy in these tumors.

1014

DELINEATION OF CHROMOSOMAL CONSENSUS REGIONS IN MANTLE CELL LYMPHOMA USING A HIGH RESOLUTION GENOMIC MICROARRAY

H. Kohlhammer,[1] S. Wessendorf,[2] D. Kienle,[2] H.A. Kestler,[3] T.F.E. Barth,[4] P. Müller,[4] G. Ott,[5] M. Bentz,[6] P. Lichter,[7] H. Döhner,[2] S. Stilgenbauer,[2] C. Schwnen[2]

[1]*Universitätsklinikum Ulm, ULM, Germany;* [2]*Abt. Innere Medizin III, Universität Ulm, Ulm, Germany;* [3]*Forschungsdozentur Bioinformatik, Ulm, Germany;* [4]*Abt. Pathologie, Universität Ulm, Ulm, Germany;* [5]*Abt. Pathologie, Universität Wurzburg, Wurzburg, Germany;* [6]*Med. Klinik II, Stdtisches Klinikum, Karlsruhe, Germany;* [7]*Abt. Molekulare Genetik, DKFZ, Heidelberg, Germany*

Mantle cell lymphoma (MCL) is a B-cell neoplasm associated with the translocation t(11;14)(q13;q32) resulting in an overexpression of cyclin D1. In addition, high numbers of secondary genomic aberrations were shown by chromosomal banding analysis, comparative genomic hybridization (CGH) and array-CGH studies. The aim of the present study was a precise delineation of chromosomal consensus regions for the most frequent genomic aberrations in MCL in order to provide a basis for the identification of candidate genes. For this purpose, a dedicated 'MCL-array' consisting of 4126 DNA-probes was developed. 21 genomic regions, known to be reccurrently affected by genomic aberrations in MCL were covered by high resolution physical maps with a total of 3359 DNA-probes. These regions encompass: 1p13-1p22, 3q24-3q29, 6p22-6p25, 6q23-6q27, 7p15-7p22, 8p21, 8q24, 9p21-9p22, 9q21-9q22, 10p12-10p15, 11q13, 11q22-11q23, 12p12-12p13, 12q12-12q21, 13q14, 13q33-13q34, 14q32, 15q25-15q26, 17p11-17p13, 18q21-18q23, 22q11-22q13. Additionally 767 probes linearly covering the genome in a distance of 4 megabasepairs were used for the normalization of the data. A first series of cryopreserved tumor samples in 25 patients with t(11;14)-positive MCLs were analyzed. In all cases, genomic aberrations were identified. The most frequent genomic gains mapped to chromosome arm 3q (14 cases) followed by gains of 7p, 11q and 18q (7 cases each). The most frequent genomic deletion affected chromosome arm 13q (18 cases). Further deletions mapped to chromosome arms 11q (12 cases), 1p and 9p (11 cases each). The smallest consensus region for genomic gains was defined for 10p13 with a size if 600 kilobaspairs, containing the BMI1 gene. The consensus region on 8q24 was narrowed down to a size of 1.0 megabasepairs, containing the MYC gene. The smallest minimal deleted regions with a size of 600 kilobasepairs each mapped to 8p21 and 9p21, containing TNFRSF10B and CDKN2A/CDKN2B. The consensus region on 12p13 was narrowed down to a size of 1.1 megabasepairs, containing CDKN1B. These data demonstrate the usefulness of a custom made high resolution microarray as a precise tool for the delineation of genomic consensus regions in MCL. Completing a larger series of MCL these data will provide a more detailed basis for the identification of altered chromatin segments, which can contribute to the identification of candidate genes in this tumorentity.

Allogeneic stem cell transplantation - Clinical

1015

LOW TREATMENT-RELATED MORTALITY AND RAPID REGRESSION OF BONE MARROW FIBROSIS AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION IN PATIENTS WITH MYELOFIBROSIS. AN INTERIM ANALYSIS OF A PROSPECTIVE STUDY OF THE CHRONIC LEUKEMIA WORKING PARTY OF THE EUROPEAN GROUP OF BLOOD AND MARROW TRANSP

N. Kröger,[1] G. Kobbe,[2] E. Holler,[2] M. Bornähuser,[2] R. Schwerdtfeger,[3] A. Nagler,[4] W. Bethge,[2] H. Wandt,[5] P. Corradini,[6] M. Stelljes,[2] J. Thiele,[2] L. Uharek,[2] J. Schubert,[2] A. Zander,[2] D. Niederwieser,[2] T. de Witte[7]

[1]*University Hospital Hamburg, Hamburg, Germany;* [2]*University Hospital, Dusseldorf, Germany;* [3]*DKD-Clinic, Wiesbaden, Germany;* [4]*Chaim Sheba Medical Center, Tel Hashomer, Israel;* [5]*Klinikum, Nurnberg, Germany;* [6]*Instituto Tumori Nazionale, Milan, Italy;* [7]*University Medical Center, Nijmegen, Netherlands*

Background. Allogeneic stem cell transplantation is the only curative approach for patients with myelofibrosis. Due to the high treatment related mortality only younger patients are candidate for this treatment approach. *Aim.* To investigated in a prospective, multicenter study the effect of a reduced-intensity conditioning regimen with busulfan (10 mg/kg), fludarabine (180 mg/mμ) and anti-thymocyte globulin, followed by allogeneic stem cell transplantation in patients with myelofibrosis *Methods*. At time of analysis (3/2006) 37 patients with related (n=16) or unrelated donors (n=21) were evaluable for toxicity, treatment related mortality and efficacy According to the Lille score, myelofibrosis was classified as low (n=7), intermediate (n=22) or high risk (n=8). The median age of the patients was 53 years (range, 32 67). Stem cell source was peripheral blood stem cells (n=36) or bone marrow (n=1) from HLA-matched donor (n=32) or HLA-mismatched donor (n=5). *Results*. No primary graft-failure was observed. The median time until leukocyte (> 1.0×10^9/L) and platelet (> 20×10^9/L) engraftment was 17 days and 22 days, respectively. The leukocyte engraftment was faster in splenectomized patients (p=0001). Acute graft-versus-host disease (GvHD) grade II IV and III/IV occurred in 21% and 8% of the cases, and 21% of the patients experienced chronic GvHD. The treatment-related mortality at one year was 6%. The cumulative incidence of relapse at three years was 18% . After a median follow-up of 12 months, the estimated three-year overall and disease-free survival was 85% and 75%, respectively. The three-year estimated disease-free and overall survival was 77% and 85%, respectively. The disease-fee survival was higher in low risk than in intermediate/high risk patients (100 vs 70%). In 20 patients, the dynamics of bone marrow changes could be monitored one month, six months and one year after stem cell transplantation by sequential bone marrow trephine biopsies. A total regression of the pre-transplant-increased fibrosis was completed in the post-transplant period after about six months while the extent of osteosclerosis did not change significantly during observation time. The CD34 progenitor cells in bone marrow were increased before transplantation but the number declined rapidly to normal values in all responding patients. Conclusions. Reduced-intensity conditioning in patients with myelofibrosis provide rapid and sustained engraftment with a low one-year treatment-related mortality resulting in an encouraging three-year overall and disease-free survival. Allogeneic stem cell transplantation results in rapid regression of fibrosis and in rapid decline of CD34 progenitor cells in the bone marrow.

1016

HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION WITHOUT *IN VITRO* T CELL DEPLETION FOR THE TREATMENT OF HEMATOLOGICAL MALIGNANCIES

X. Huang, D.H. Liu, K.Y. Liu, H. Chen, W. Han, Y.H. Chen, J.Z. Wang, Z.Y. Goa, Y.C. Zhang, Q. Jiang, H.X. Shi, D.P. Lu

Peking University Institute of Hematolog, Beijing, China

Backgrounds. Many patients who require allogeneic hematopoitic stem cell transplantation (allo-HSCT) do not have a human leukocyte antigen (HLA)-matched donor. HSCT from HLA-mismatched family donors is associated with lower long-term survival and delayed immune reconstitution, especially if the T cells are depleted. *Aims*. Here we describe a method for haploidentical allo-HSCT from family members without *in vitro* TCD, designed to overcome the HLA barrier and reduce transplant-related complications. *Results*. In the present study, the method for haploidentical allo-HSCT without *in vitro* TCD involves sequential, in vivo modulation of T cell functions in the recipient and the donor, and adjustment of the dose of donor HSCs. This protocol has three elements: anti-human thymocyte immunoglobulin (ATG) for the prevention of GVHD, a combination of G-PBSCs and G-BM, and donor treatment with recombinant human G-CSF. There are 176 patients, including 88 with high-risk malignancies, underwent transplantation from an HLA-haploidentical family donor with 1-3 mismatched loci. All patients achieved sustained, full donor chimerism. The cumulative incidence for to acute GVHD was 22.7%, which was not associated with HLA disparity. The cumulative incidence for extensive cGVHD was 46.9%. The 2-year probability of relapse was 12.2% in the standard risk group and 38.9% in high-risk group. The probability of 1-year and 2-year leukemia-free survival (LFS) was 79.1% and 68.2% for standard-risk patients and 54.3% and 42.1% for high-risk patients (p=0.0009) respectively. Summary/Conclusion: These results show that G-BM combined with G-PBSCs from haploidentical family donors, without *in vitro* TCD, could be used as a good source of stem cells for allo-HSCT. The new HSCT regimen described here allows use of a haploidentical family members as donors, a strategy likely to be much more important in the future, for the increasing numbers of Chinese patients, and those of other ethnicities, who are the only child in the family.

1017

PURIFIED T DEPLETED PERIPHERAL BLOOD AND BONE MARROW CD34 TRANSPLANTATION FROM HAPLOIDENTICAL MOTHER TO CHILD WITH THALASSEMIA

B. Erer,[1] P. Sodani,[1] A. Isgr,[1] J. Gaziev,[1] P. Polchi,[1] A. Roveda[1], F. Pagliai,[1] M. Andreani,[1] L. Faulkner,[2] V. Tintori,[2] G. Adorno,[3] A. Lanti,[3] G. Isacchi,[4] F. Zinno,[4] G. Lucarelli[1]

[1]*Mediterranean Institute of Hematology, Rome, Italy;* [2]*Paediatric Hospital Meyer, Florence, Italy;* [3]*SIMIT Tor Vergata, Rome, Italy;* [4]*SIMIT Paediatric Hospital Bambino Gesù, Rome, Italy*

Approximately 60% of thalassemic patients can not apply to 'gene therapy today' which the insertion of one allogenic HLA identical stem cell into the empty bone marrow as the vector of the normal gene for β globin chain synthesis. We studied the use of the haploidentical mother as the donor of hematopoietic stem cells assuming that the immunotollerance established during the pregnancy will help to bypass the HLA disparity and allow the hemopoietic allogeneic reconstitution in the thalassemic recipient of the transplant. We have employed a new preparative regimen for the transplant in fourteen thalassemic children aged 3 to 12 years (median age 5 years) using T cell depleted peripheral blood stem cell (PBSCTs) plus bone marrow (BM) stem cells.. All patients received hydroxyurea (OHU) 60 mg/kg and azathioprine 3 mg/kg from day -59 until day-11, fludarabine (FLU) 30 mg/m 2 from day -17 to day -11, busulphan (BU) 14 mg/kg starting on day -10, and cyclophosphamide(CY) 200mg/kg, Thiotepa 10 mg/kg and ATG Sangstat 2.5 mg/kg, followed by a CD34 + t cell depleted (CliniMacs system), granulocyte colony stimulating factor (G-csf) mobilized PBSC from their HLA haploidentical mother. The purity of CD34+ cells after MACS sorting was 98-99%, the average number of transplanted CD34+ cells was 15, 4 x 10 6/kg and the average number of infused T lymphocytes from BM was 1,8 x 10 5/Kg.The patients received cyclosporin after transplant for graft versus host disease(GVHD) prophylaxis during the first two months after the bone marrow transplantation. Results. All patients are alive.Three patients rejected the transplant and are alive with thalassemiaTwo patients engrafted after a second transplantation. Eleven\ patients are alive disease free with a median follow up of 26 months (range 7-42).None of the seven patients showed AGVHR. This preliminary study suggest that the transplantation of megadose of haploidentical CD34+ cell from the mother is a realistic therapeutic option for those thalassemic patients without genotipically or phenotipically HLA identical donor.

1018

INCREASED $CD4^+CD25^{HIGH+}$ REGULATORY T-CELL ARE ASSOCIATED WITH DISEASE RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR CHRONIC MYELOID LEUKAEMIA (CML)

E. Nadal, J. Kaeda, J.F. Apperley, R. Lechler, F. Dazzi

Imperial College, London, United Kingdom

Backgrounds. The success of SCT after CML largely relies on the graft versus leukemia (GvL) effect exert by donor T-cells. $CD4^+CD25^+$ regulatory T-cell (Tregs) play a crucial role in the maintenance of peripheral tolerance and have been tested in animal models to successfully prevent GVHD. The role of Tregs in clinical transplantation remains unclear, insofar as the few studies published to date have reported controversial

results regarding GvHD. Although there is emerging evidence that Tregs are associated with a poor outcome in cancer patients, none of these studies has investigated the role of Tregs in leukaemia relapse post-SCT. *Aims.* To quantify CD4CD25high regulatory T-cells in post-SCT patients and correlate their levels with clinical outcome. *Materials and Methods.* We performed a cross-sectional study at a single institution. We enumerated and characterised peripheral blood $CD4^{+}CD25^{high+}$ Tregs in 76 patients after allogeneic SCT for CML by FACS analysis. As control we analysed 21 samples from healthy volunteers and 20 samples from newly diagnosed CML patients. BCR-ABL/ABL ratio was determined in every sample by real-time PCR. Patients were considered in remission if the ratio was less than 0.02% and in relapse if higher. All quoted p-values are two-sided with $p<0.05$ considered statistically significant. *Results.* Patients after SCT had higher levels of Treg than normal donors (median 1.5% vs 0.87, $p<0.01$) and untreated CML (median 1.5% vs 0.27, $p<0.0001$). In the multiple regression analysis only the time post SCT (before or after 18 months) and disease status (molecular remission versus relapse) were predictive for increased Tregs (Coef -2.994, $p=0.004$ and Coef -2.395, $p=0.020$ respectively). No association with Treg levels and GvHD was found. The logistic regression analysis performed in 43 patients that had not received DLI post SCT confirmed that increased Tregs, both as percentage or absolute numbers, were the only predictive variable for relapse (exp 1.44, $p=0.011$). *Conclusions.* A substantial expansion of Tregs occurs early after allogeneic SCT and the presence of high numbers of Tregs 18 months after transplant is predictive of leukaemic relapse. Although the increment might initially have an advantageous effect on graft rejection, our data suggest that Tregs exert an inhibitory effect on GvL.

1019

CHRONIC GVHD HAS A ROLE IN MAINTAINING REMISSIONS AFTER IMATINIB IS DISCONTINUED IN PH+ALL PATIENTS TRANSPLANTED IN CR1

B. Wassmann,[1] H. Pfeifer,[1] M. Stadler,[2] M. Bornähuser,[2] G. Bug[1], M. Stelljes,[2] R. Schwerdtfeger,[3] N. Basara,[4] U. Hegenbart,[2] R. Mahlberg,[5] D. Hoelzer,[1] O.G. Ottmann[1]

[1]*University Hospital Frankfurt, Frankfurt/Main, Germany;* [2]*University Hospital, Hannover, Germany;* [3]*Deutsche Klinik fr Diagnostik, Wiesbaden, Germany;* [4]*Klinik fr Knochenmarktransplantation, Idar-Oberstein, Germany;* [5]*Mutterhaus der Borromerinnen, Trier, Germany*

Backgrounds. Reappearance of BCR-ABL transcripts after allogeneic stem cell transplantation for Ph+ALL indicates evolving relapse. Intervention with imatinib may be associated with renewed and sustained PCR negativity. However, the clinical consequences of discontinuing imatinib are not known. *Methods.* We updated a previously reported prospective study of post-transplant imatinib for molecular relapse to assess the outcome of patients in whom imatinib was discontinued after sustained PCR negativity. *Results.* The present analysis includes exclusively the subset of 14 patients in whom BCR-ABL transcripts became undetectable shortly after (median: 46d, range: 27-111d) initiation of imatinib. Fourteen of 15 patients who did not achieve early PCR negativity have relapsed. When imatinib was discontinued after 12.2 (range: 1.4-17.5) months, BCR-ABL transcripts were undetectable in 13 of the 14 patients who had achieved PCR negativity. A median of 16.5 (range 3.3-39.4) months after stopping imatinib, 6 of these 14 patients were PCR negative and alive, one experienced reappearance of BCR-ABL transcripts and is currently treated with imatinib and donor lymphocyte infusions (DLI), 3 patients died in molecular CR, and 3 patients relapsed (CNS:n=1, BM:n=2) 3.3, 16.4 and 13.6 months after imatinib discontinuation. One patient converted to PCR positivity while still on imatinib and was entered in a clinical trial of AMN107 in conjunction with DLI. None of the 8 patients with active chronic GvHD at imatinib discontinuation relapsed either at the molecular level or hematologically, with a median follow-up of 16 (range 6.2-32.9) months. Estimated probability of remission 24 months after discontinuation of imatinib in patients with and without chronic GvHD was 100% and 40%, respectively ($p=0.04$). Conclusion: We conclude that it is appropriate to discontinue imatinib in patients who previously underwent allogeneic SCT, remained PCR negative on imatinib for approximately one year, and have ongiong chronic GvHD. Absence of GvHD is associated with a higher risk of relapse following termination of imatinib therapy.

Platelets and bleeding disorders

1020

INCIDENCE, CLINICAL-LABORATORY FEATURES AND MANAGEMENT OF ACQUIRED VON WILLEBRAND SYNDROME AND OTHER ACQUIRED DEFECTS OF HEMOSTASIS IN A COHORT OF 240 PATIENTS WITH CHRONIC LYMPHO-MYELOPROLIFERATIVE DISORDERS

A.B. Federici,[1,2] P. Bucciarelli,[3] M.T. Canciani,[3] F. Gianniello,[3] B. Moroni,[3] A. Artoni,[3] R. Bader,[3] P.M. Mannucci[3]

[1]*University of Milan, Milan, Italy;* [2]*A. B. Bonomi Hemophilia Thrombosis Ctr, Milan, Italy;* [3]*Department of Internal Medicine, Milan, Italy*

Background. Acquired von Willebrand Syndrome (AVWS) is a rare bleeding disorder with laboratory findings similar to those for congenital von Willebrand disease. The actual prevalence of AVWS in the general population is unknown because large prospective studies on this syndrome are not available. Retrospective data showed that AVWS is especially frequent in lympho- (LPD) or myeloproliferative (MPD) disorders. *Aims and design of the study*. to determine incidence, clinical-laboratory features and management of AVWS and other acquired hemostatic defects, we have sequentially observed for one year our cohort of patients with chronic LPD/MPD. Exclusion criteria were platelet counts <70,000/uL and any therapies, including non-steroid anti-inflammatory drugs. *Methods.* A bleeding severity score derived from a detailed history of 11 symptoms. Screening tests: bleeding time (BT), prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT) and, if prolonged, PT-PTT-TT 50:50 mixing tests. Additional specific tests: FVIII/VWF activities (AVWS/HA); platelet nucleotides (acquired storage pool defects, ASPD); silice clotting time (SCT), Russel viper venom time (RVVT), anticardiolipin antibodies (ACA) for lupus anticoagulant-antiphospholipid antibodies (LAC/APA). *Results.* Among 458, 240 patients satisfied the inclusion criteria, with percentual (%) diagnosis of MGUS (38), ET (38), CLL (7), PV-CML-IMF (7), HD-NHL (5), MDS M (2), MM (2) and amyloidosis (1). Results are:

Features	*Lymphoproliferative*	*Myeloproliferative*	*Total*
Case number (%)	122 (51)	118 (49)	240 (100)
Bleeding score (> 10)	30/122 (25)	18/118 (15)	48/240 (20)
Abnormal screening tests	57/122 (48)	22/118 (19)	79/240 (33)
Acquired defects	21/122 (17)	38/118 (32)	59/240 (25)
1) AVWS	10/122 (8)	12/118 (10)	22/240 (9)
2) ASPD	0/ 122 (0)	19/118 (16)	19/240 (8)
3) LAC/APA	8/ 122 (7)	3/118 (3)	11/240 (5)
4) anti FVIII or X inhibitors	3/122 (2)	4/118 (3)	7/240 (3)

In one year, severe mucosal (n=21) and non-mucosal (n=13) bleeds in LPD (n=12) or MPD (10) were treated with DDAVP (n=18), FFP/concentrates (n=4), IVIg (n=10), rFVIIa (n=2). *Conclusions.* AVWS and the other acquired hemostatic defects shown here are not so rare (9/16%) and can be severe in LPD/MPD. An early correct diagnosis should improve morbidity and mortality of patients with bleeding complications in chronic LPD/MPD.

1021

DIFFERENT ALLELIC DISTRIBUTION OF SINGLE NULCEOTIDE POLYMORPHISMS AT CODONS 10 AND 25 OF TGFB IN A GROUP OF 122 HHT ITALIAN PATIENTS AND CONTROLS

M. Martinetti,[1] A.M. Iannone,[1] C. Olivieri,[2] C. Badulli,[1] E. Sanzani,[2] F. Pagella,[1] L. Salvaneschi,[1] E. Buscarini,[3] C. Danesino[2]

[1]*IRCCS Policlinico S. Matteo, Pavia, Italy;* [2]*Genetica Medica, Universit degli Studi, Pavia, Italy;* [3]*Gastroenterologia Ospedale Maggiore, Crema, Italy*

Hereditary hemorrhagic telangiectasia (HHT) (*OMIM 187300*) is an autosomal dominant vascular disorder characterized by telangiectases on skin and mucosae (causing epistaxes and gastrointestinal bleeding, that may be severe enough to require transfusions) and visceral arterovenous malformations (AVMs). Epistaxes and telangiectases are the most frequent symptoms, present in more than 95% of the patients. AVMs

are mostly observed in liver, lungs and brain and may cause severe life-threatening complications. The phenotype is highly variable, even among members of the same family, and penetrance is usually complete by the age of 40 years. About 80% of HHT patients carry mutations in either of two genes: ENG (OMIM #131195) (HHT1) or ACVRL1 (OMIM #601284) (HHT2) which code for a TGFb receptor type III and I respectively. Evidence for a third locus has also been reported. Association of the HHT phenotype with Juvenile Polyposis and mutations in the MADH4 gene (also involved in the TGFb signalling pathway) have recently been demonstrated as well. TGFb is a multifunctional cytokine which modulates a wide spectrum of biological activities, including cell proliferation, differentiation, and adhesion as well as extracellular matrix formation. TGFB1 gene contains many polymorphisms, mostly SNPs, some of which affect its transcription, causing individual variations in protein production. *Aims*. To assess the genotype distribution of *TGFb* codon 10 and 25 polymorphisms (which are known to be related to protein production levels) in a group of 122 patients (58 index cases) affected with HHT in whom the causing mutation in *ENG* or *ACVRL1* was known. *Methods*. A 500 bp fragment of *TGFb* gene including codons 10 and 25 was amplified by PCR and subsequenly digested by MspAI (codon 10 SNP) and FseI (codon 25 SNP) enzymes. Digested products were analysed on polyacrilamide 7% and agarose 3% gels respectively. A subgroup of 20 patients was genotyped by PCR-SSP using the cytokine typing kit provided by Pel Freez Company. Statistical analysis was performed using the χ^2 test (202 controls). *Results*. A statistically significant difference in the distribution of codon 10 and 25 was observed; for codon 10, it was limited to the subgroup carrying the ACVRL1 mutation, while the allele distribution at codon 25 was more widely different from controls. This skewed distribution leads to statistically significant differences in the TGFb *producer genotypes* between controls and HHT patients: in this last group, in fact, there is a higher than expected percentage of intermediate and low producers (p>0.01). *Summary.* HHT is a vascular autosomal dominant disorder characterised by nosebleeding, telagiectases and AVMs. A wide inter and intra-familial variability in the phenotype is present. The genes involved (ENG and ACVRL1) belong to the TGFb signalling pathway and we assessed genotype distribution of two TGFb SNPs (codons 10 and 25) related to the protein production in a group of 122 HHT patients with a known causing mutation. Statistically significant results were obtained for codon 25 and some codon 10 subgroups. We suggest that differences in TGFb production can partially explain the phenotypic variations.

1022

ELTROMBOPAG INCREASES PLATELETS DURING 6-WEEK TREATMENT OF ITP RESULTS OF A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE II STUDY

A. Newland,[1] L. Stone,[2] G. Cheng,[3] M. Saleh,[4] L. Kovaleva,[5] J. Bussel,[6] H. Hassani,[2] N. Stone,[2] A. Provan[2]

[1]*The Royal London Hospital, London, United Kingdom;* [2]*GlaxoSmithKline, Collegeville, PA, USA;* [3]*Prince of Wales Hospital, Shatin, Hongkong (China);* [4]*Georgia Cancer Specialists, Atlanta, GA, USA;* [5]*Hematology Research Center, Moscow, Russian Federation;* [6]*Weill Medical College of Cornell Univ, New York, NY, USA*

Background/Aims. Eltrombopag olamine, a novel, small molecule, oral platelet growth factor was studied in a global, randomized, double-blind, placebo-controlled phase II trial in adult patients with chronic idiopathic thrombocytopenic purpura (ITP) and platelet counts <30,000/μL. *Methods.* The primary efficacy endpoint was the proportion of patients with platelets greater than or equal to 50,000/microL after 42 days of dosing using last observation carried forward. Randomization was stratified by splenectomy status, use of concomitant ITP therapy and platelet counts less than or equal to 15,000/μL. *Results.* One hundred and four patients were randomized into placebo (N=26), 30mg (N=27), 50mg (N=27) and 75mg (N=24) eltrombopag arms. The majority of patients were females (62%) and of Caucasian origin (70%). Prior treatment of ITP included corticosteroids (73%), IVIg (37%) and anti-D (27%). During the study 35 (34%) patients received concomitant ITP therapy. At Day 43, a dose dependent increase in the proportion of responders (platelet count greater than or equal to 50,000/μL) was observed: placebo (16%), 30 mg (28%), 50 mg (67%) and 75mg (86%). The odds-ratio of treatment response to placebo was statistically significant in the 50 and 75mg arms (p<0.001). Similar efficacy response of eltrombopag relative to placebo was observed regardless of strata (splenectomy status, concomitant ITP treatment and baseline platelet count). The percentage of patients achieving a platelet count >200,000/μL was: placebo (4%), 30mg (12%), 50mg (38%) and 75mg (52%). The median platelet count on Day 43 was 16, 29, 132 and 202,000/μL for placebo, 30mg, 50mg and 75mg eltrombopag, respectively. Overall, the safety profile was similar across the treatment groups, with the following percentage of patients experiencing at least one adverse event (AE) during treatment: placebo (58%), 30 mg (44%), 50 mg (44%) and 75 mg (58%). Headache, AST elevation, constipation and epistaxis were the only AEs occurring in greater than or equal to 5% of patients in the eltrombopag arms. The most common AE in the placebo arm was fatigue, occurring in 19% of patients, compared to 3% of all patients exposed to eltrombopag. The most common AE in the eltrombopag 75 mg arm was headache (21%), compared to 15% of patients exposed to placebo. A total of 2(8%) placebo and 2(7%) 50mg patients experienced at least one serious AE (SAE) during treatment; of which, 1(4%) placebo patient and 1(4%) 50 mg patient experienced at least one related SAE during treatment. No SAEs were reported during the treatment period on the 30 mg and 75mg eltrombopag arms. No other dose dependent safety concerns were identified. *Summary/Conclusions.* Eltrombopag at doses of 50 and 75mg significantly increased platelet counts during the 6 week treatment period compared to placebo. No dose dependent safety concerns were identified. Phase III trials of eltrombopag in patients with ITP are ongoing.

1023

GENETIC DEFECTS CAUSING TYPE 3 VON WILLEBRAND DISEASE IN HUNGARY

A. Mohl,[1] Z. Boda,[2] R. Jáger,[3] H. Losonczy,[4] A. Marosi,[5] T. Masszi,[6] E. Nagy,[1] L. Nemes,[6] T. Obser,[7] F. Oyen,[7] G. Radványi[8], A. Schlammadinger,[2] Z.S. Szélessy,[6] K. Vezendy[9], R. Schneppenheim,[7] I. Bodó[6]

[1]*Semmelweis University, Budapest, Hungary;* [2]*Debreceni Egyetem, Debrecen, Hungary;* [3]*National Blood Bank Service, SZOMBATHELY, Hungary;* [4]*Pcsi Tudomnyegyetem, PCS, Hungary;* [5]*Heim Pl Hospital, Budapest, Hungary;* [6]*National Medical Center, Budapest, Hungary;* [7]*University Medical Center, Hamburg, Germany; 8Semmelweis Hospital, Mioskolc, Hungary; 9Szegedi Tudomnyegyetem, Szeged, Hungary*

Backgrounds. Type 3 VWD, an autosomal recessive severe bleeding disorder, is characterized by very low to undetectable plasma von Willebrand factor (VWF) and, consequently, reduced plasma factor VIII levels. Genetic mutations responsible for type 3 VWD are very heterogenic, and show variability among different patient populations. With the exception of 2435delC, repeatedly found in populations adjacent to the Baltic Sea, no prevalent mutation has been found in populations so far studied. *Aims.* We set out to characterize the genotype of type 3 VWD in the entire Hungarian population of ten million. *Methods.* We studied the genetics of 27 patients by direct sequencing of all 52 exons of the von Willebrand factor gene. *Results.* The prevalence of the disease in Hungary is 0.29/100.000. Several novel nonsense and frame shift mutations were identified: Q1238*, Q1898*, Q1931*, S2568*, S2505* and 1993insC, 2124delCT, 3622delT, 5316insGA, 7333delG. Previously described mutations found in Hungarian patients were: R1659*, R1853* and 2269delCT. In addition, a large partial deletion at the 5' end of the gene was also identified. 2435delC in exon 18, the single most frequent deletion in other populations of Europe, was detected in 6 patients (allele frequency among patients approximately 13%). Finally, three novel missense mutations and two possible splice site mutations were also detected: C35R, R81G, C623T and 3379+1G→A (reported previously), 1730-10C→A. Summary: Type 3 VWD is caused by heterogeneous mutations scattered throughout the entire gene. If confirmed by a detailed comparison to other populations, the above new mutations suggest that the Hungarian type 3 VWD patient population may be genetically distinct.

1024

THE LOW MOLECULAR WEIGHT TPOR AGONIST, ELTROMBOPAG, DOES NOT PRIME PLATELET ACTIVATION *IN VITRO*

A. Erhardt, M. Abboud, J. Toomey, C. Erickson-Miller

GlaxoSmithKline, Collegeville, USA

Backgrounds. Eltrombopag is a non-peptidyl small molecule thrombopoietin receptor (TpoR) agonist currently in clinical development for the treatment of patients with thrombocytopenia. Stimulation of this receptor results in enhanced megakaryocyte proliferation, differentiation, and ultimately platelet production. In addition to effects on megakaryocytes, TpoR activation via thrombopoietin (TPO) is known to directly stimulate platelet function. The physiological consequences of platelet stimulation in this setting are unclear; however, it could represent a general liability in the utilization of TpoR agonists. *Aims.* The objective of the present study was to compare the direct platelet activating potential of eltrombopag to TPO *in vitro*. *Methods.* Platelets were obtained from healthy volunteers, and the activation of signal transduction pathways was examined in washed platelet preparations. Platelet aggregation was examined under multiple experimental conditions, including washed platelet preparations and platelet-rich-plasma (PRP) anticoagulated with either citrate or hirudin. Platelet α-granule release was determined via FACS measurement of CD62P. *Results.* In signal transduction studies of washed human platelets, TPO activated Stats-1,3,5 and Akt. In comparison, eltrombopag partially activated Stats-3 and 5, with no/minimal activation of Stat-1 or Akt. In platelet aggregation studies, TPO acted in synergy with subthreshold/submaximal concentrations of ADP or collagen to induce maximal aggregation under all conditions examined. In contrast, eltrombopag induced weak and inconsistent activation of washed platelets; however, no synergy was observed when examined in PRP. Similar to aggregation results, platelet activation as examined via surface expression of CD62P was significantly enhanced by TPO as compared to eltrombopag. *Conclusions.* The present study demonstrates that the TpoR agonist eltrombopag has only a limited capacity to induce human platelet activation, suggesting that potential platelet activation liabilities associated with peptidyl TpoR agonists could be attenuated via a small molecule approach.

Philadelphia chromosome positive leukemias

1025

DOMINANT ROLE OF PRE-TREATMENT BCR-ABL KINASE DOMAIN MUTATIONS IN ELDERLY *DE NOVO* PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH+ALL) PATIENTS DEVELOPING RESISTANCE TO IMATINIB-BASED TREATMENT

H. Pfeifer,[1] B. Wassmann,[1] A. Pavlova,[2] T. Lange,[3] L. Wunderle[1], P. Brück,[1] M. Müller,[4] J. Oldenburg,[2] A. Hochhaus,[4] D. Hoelzer,[1] O.G. Ottmann[1]

[1]*Universitätsklinik Frankfurt, Frankfurt, Germany;* [2]*Blutspendedienst Hessen, Frankfurt/Main, Germany;* [3]*Universitätsklinik Leipzig, Leipzig, Germany;* [4]*Klinikum Mannheim, Mannheim, Germany*

Backgrounds. BCR-ABL tyrosine kinase domain (TKD) mutations are an important cause of acquired imatinib resistance in Ph+ALL patients who previously failed chemotherapy or stem cell transplantation. In some of these patients, identical mutations have been detected prior to and at relapse on imatinib, suggesting selection of resistant mutant clones. In patients with newly diagnosed Ph+ALL receiving front-line imatinib-based combination therapy, the role of TKD mutations in imatinib resistance has not been determined. *Patients and Methods.* Using denaturing high-performance liquid chromatography (D-HPLC) and cDNA sequencing, we retrospectively analyzed for BCR-ABL mutations 232 bone marrow and 109 peripheral blood samples from 51 elderly patients (median age 68 yrs.) with *de novo* Ph+ALL who were enrolled in a prospective, randomized clinical trial comparing imatinib and chemotherapy as induction treatment, followed by consolidation with imatinib in combination with chemotherapy. Analyzed samples were collected prior to or up to the end of induction therapy, at the time of relapse or in case of ongoing CR at the last time point with detectable bcr-abl transcripts. Results of D-HPLC and sequencing were assessed in parallel by allel-specific oligonucleotide (ASO) - PCR assay for the T315I mutation. *Results.* TKD mutations were detected in 20 of the 40 patients who were evaluable pre-study or up to the end of induction therapy, while 20 samples had wild-type bcr-abl. In all but one pre-imatinib sample with a mutation, this was detected only at a low level. CR rate in patients randomly assigned to imatinib induction was 90% in patients with and without detectable mutations pre-study. The probability of bcr-abl transcripts becoming undetectable during the course of therapy in patients with or without a mutation was 40% and 37%, respectively. Median remission duration in pts. with a T315I mutation (n=4) was 130 d (range: 53-319d), whereas it was longer in patients with an activation loop (526 d; range: 504-549d) or P-loop mutation (411 d; 106-745d). To date, 7 patients with an initially detected mutation remain in CR (median FU: 12,8 mo., range 2,4 - 24,5 mo.). Conversely, 3 of the 20 (15%) patients without a pretherapeutic mutation displayed a mutation at relapse, 4 patients (20%) showed no mutation at relapse. There was no significant difference in overall remission duration at 18 months in patients with any or without pre-therapeutic mutations (10.6 months versus 13 months; p=n.s.). At relapse, a TKD mutation was identified in 83% of patients (P-loop 70%, T315I 20%, A-loop 10%, double mutation 10%); the D-HPLC pattern indicates concordance between the mutation detected in pre-therapeutic specimens and the dominant mutation detected at relapse. *Conclusions.* Bcr-abl TKD mutations conferring high-level resistance to imatinib are the predominant cause of acquired resistance to imatinib in elderly pts. with *de novo* Ph+ALL. Mutations identified at relapse are already detectable prior to or during the induction phase in approximately half of patients, almost always at very low frequency. Efforts to improve treatment outcome in Ph+ALL will need to focus on the identification and elimination of TKD mutations during early stages of treatment.

1026

RANDOMIZED COMPARISON OF IMATINIB WITH IMATINIB COMBINATION THERAPIES IN NEWLY DIAGNOSED CHRONIC MYELOGENOUS LEUKEMIA PATIENTS: DESIGN AND FIRST INTERIM ANALYSIS OF A PHASE III TRIAL

F.G. Guilhot,[1] P. Rousselot,[2] P. Cony-Makhoul,[3] J.J. Sotto,[4] B. Rio,[5] H. Guy,[6] H. Tilly,[7] M. Schoenwald,[8] J.J. Kiladjian,[9] E. Jourdan,[10] C.E. Bulabois,[11] B. Christian,[12]

[1]University Hospital, Poitiers, France; [2]Hopital saint Louis, Paris, France; [3]Centre Hospitalier Region Annecienne, Annecy, France; [4]CHU Grenoble, Grenoble, France; [5]Hotel Dieu, Paris, France; [6]CHU Le Bocage, Dijon, France; [7]Centre henri Becquerel, Rouen, France; 8Hopital La Source, Orleans, France; 9Hopital Avicenne, Bobigny, France; [10]Hopital Caremeau, Nimes, France; [11]CHU Besancon, Besancon, France; [12]Notre Dame de Bon Secours, Metz, France

Backgrounds. Imatinib (IM) at 400 mg daily has emerged as the preferred therapy for newly diagnosed CML pts. Despite impressive results, only a minority of pts treated with IM achieved a molecular remission. To improve upon these results, the CML French Group designed a phase III, multicentre, open-label, prospective randomized trial. *Methods.* The experimental arms are IM 400 mg daily in combination with Peg-IFNα 2a, 90 μg weekly or IM 400mg daily in combination with Ara-C, (20 mg/m^2/day, days 15-28 of 28-day cycles) or IM 600mg daily. The reference arm is IM 400mg daily. All pts (over 18 years of age with Bcr-Abl positive CML in chronic phase within 3 months of diagnosis) receive IM 400 mg/day as monotherapy days 1-14 and then start the assigned randomized regimen. Treatment continues at least 12 months or until treatment failure or major toxicity. The primary endpoint will be the overall survival. Other endpoints will be: rate and duration of hematologic and cytogenetic responses, major (MCyR) and complete (CCyR), molecular response and the tolerability. Using treatment allocation ratio 1.1.1.1, randomization is stratified according to Sokal risk groups. An interim analysis of the first 636 patients (α=0.85%, β=10%) at 1 year from randomization will allow evaluation of molecular response rates, one of the experimental arm being selected for further comparison with IM 400. The increased dose of IM or a combination regimen would be considered as promising if it increased the 4 log reduction response rate by at least 20 percentage points, e.g. from 15% to 35%, with an acceptable tolerability. Evaluation of molecular response up to 12 months is centralized and blinded. *Results.* This evaluation is based on a cohort of 315 pts with a median time of observation of 12 months, [median age 53 yrs (18-78), 60% of pts were male; Sokal risk distribution: 39% of pts low risk, 38% intermediate risk, and 23% high risk]. At 3 months 82% of pts achieved complete hematologic response. Cytogenetic data are available from 154 pts. At 6 months, 135 pts (87%) achieved a MCyR, being complete in 105 pts (68%). Grade 3/4 neutropenia and thrombocytopenia occurred in 5% and 0% of IM400 pts , in 5% and 1% of IM600 pts, in 30% and 4% of IM+IFN pts and in 24% and 13% of IM+Ara-c pts respectively. Dose of Peg IFN was reduced in 16% of pts, 45 μg per week being well tolerated. Grade 3/4 non hematological toxicity occurred in 6% of IM400 pts (mainly skin rash and muscle cramps), in 10% of IM600 pts, in 5% of IM+IFN pts (maily skin rash) and in 13% of IM+Ara-c pts (mainly diarrhea). Discontinuation of experimental treatment occurred in 15% of IM600 pts, 28% of IM+IFN pts and in 13% of IM+Ara-c pts. *Conclusions.* This first analysis has proven feasibility of IM combinations in addition to high response rates. However a substantial hematological toxicity was recorded with IFN or Ara-c combination, which requires a careful assessment during the first 6 months of treatment.

1027

A FLOW-CYTOMETRIC IMMUNOBEAD ASSAY FOR THE DETECTION OF BCR-ABL FUSION ONCOPROTEIN IN PRECURSOR-B-ALL

F. Weerkamp, Y.Y. Ng, K.A.J. Brouwer-de Cock, E.E.L. Koster, T. Schonewille, V.H.J. van der Velden, F.J.T. Staal, J.J.M. van Dongen

Erasmus MC, Rotterdam, Netherlands

The BCR-ABL fusion gene, caused by t(9;22), is a frequent chromosomal aberration in precursor B-ALL patients (25-40% of adults cases, 3-5% of pediatric cases) and is associated with a poor prognosis, requiring a high-intensity treatment protocol. Rapid detection of the BCR-ABL translocation in precursor-B-ALL patients at diagnosis is therefore important for the choice of treatment. Current approaches for the detection of the BCR-ABL fusion gene employ karyotyping, FISH or PCR. However, these techniques take relatively long and demand a specialized laboratory. Our aim was to develop a flow cytometric bead-based assay (CBA) that detects the BCR-ABL fusion gene product in an easy, rapid and accurate manner. In this assay, a bead-bound catching antibody recognizes one part of the fusion protein, whereas a biotin-conjugated detection antibody recognizes the opposite part of the fusion protein. Only when a lysate of patient cells contains the fusion protein, immunobeads will give a positive signal in the flow cytometer. We generated a novel antibody specifically recognizing the exon 1-encoded domain of the BCR protein, using a developmental strategy and screening method that increases the likelihood of producing antibodies that are suitable for flow cytometry. The anti-BCR antibody was coupled to BD CBA Flex beads. After incubation of these beads with cell lysate, an already existing biotinylated anti-ABL antibody was used as detection antibody together with streptavidin-PE. Using this bead assay, we detected a strong PE signal in cell lysates of the cell lines K562, LAMA-84 (both p210), TOM-1 (p190) and AR230 (p230) harboring the BCR-ABL translocation, but not in cell lines with other translocations or normal PBMCs. A robust signal could be detected with less than 1% of LAMA-84 cells in a background of normal PBMCs, showing the high sensitivity of the assay. We then tested the assay on lysates of precursor-B-ALL patients and showed a highly specific signal only in patients that were tested positive for the BCR-ABL fusion gene using standard PCR and/or FISH techniques. We conclude that this novel bead assay can be used for diagnosis of precursor-B-ALL patients. The new assay has major advantages over currently used techniques. It is fast (completed within a few hours) and can easily be performed in a standard diagnostic laboratory using routine flow cytometry. The assay is accurate and sensitive and as recognition does not involve the break-point region, all different BCR-ABL protein variants (p190, p210 and p230) can be detected. Furthermore, as this assay involves BCR-ABL protein rather than RNA or DNA, it will measure the presence of cells that are sensitive to imatinib therapy and may be used for monitoring of treatment effectiveness.

1028

A PHASE II STUDY OF NILOTINIB (AMN107), A NOVEL INHIBITOR OF BCR-ABL, ADMINISTERED TO IMATINIB RESISTANT AND INTOLERANT PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA IN CHRONIC PHASE (CP)

P. le Coutre,[1] N. Gattermann,[2] S.G. O'Brien,[3] K. Bhalla,[4] A. Hochhaus,[5] F. Cervantes,[6] T. Rafferty,[7] L. Alland,[7] O. Ottmann[8], F. Giles[9], H. Kantarjian[9]

[1]Campus Virchow Charit, Berlin, Germany; [2]University of Duesseldorf, Duesseldorf, Germany; [3]Royal Victoria Infirmary, University Newcastle, United Kingdom; [4]H. Lee Moffitt Cancer Center, Tampa, USA; [5]University of Heidelberg, Mannhein, Germany; [6]Hospital Clinic-Provincial, Barcelona, Spain; [7]Novartis Pharmaceuticals Florham Park, New Jersey, USA; [8]University of Frankfurt, Frankfurt A M, Germany; [9]MD Anderson Cancer Center, Houston, USA

Backgrounds. Nilotinib is a potent, highly selective, aminopyrimidine inhibitor which *in vitro* is 30-fold more potent than imatinib and active against 32/33 imatinib resistant Bcr-Abl mutations. Aim: This study was designed to evaluate the safety and efficacy of Nilotinib as defined by hematologic/cytogenetic response (HR/CyR) rates in imatinib-resistant or intolerant CP patients. Methods. This study is a Phase II, open-label, multi-center study of nilotinib administered at an oral dose of 400 mg twice daily. *Results.* Preliminary data are reported for 67 patients including 39 (58%) with imatinib-resistant and 27 (40%) imatinib-intolerant CML (1 pt unknown). Median age was 62 (range 31-80) yrs and the median time from diagnosis to treatment was 52 (range 5-279) months. BCR-ABL mutations associated with imatinib resistance were detected in 11/17 (65%) patients at baseline. The median duration of nilotinib exposure was 129 (range 3-225) days. Treatment is ongoing for 50 (75%) patients with 17 (25%) patients discontinued (9 adverse events, 2 disease progression, 6 other). Major CyR was observed in 13 (19%) patients (6 complete, 7 partial), minor CyR was observed in 4 (17%) patients, and minimal CyR in 5 (7%) patients. Complete HR was reported in 35 of 42 (83%) patients without a CHR at baseline. Adverse events occurring in 10% of patients were headache 36% (n=24), fatigue 31% (n=21), pruritus 28% (n=19), rash, nausea, diarrhea 27% (n=18 each), constipation 16% (n=11), thrombocytopenia, anemia, vomiting 15% (n=10 each), neutropenia 13% (n=9), bone pain, muscle spasms, arthralgia, peripheral edema 12% (n=8 each), abdominal pain, myalgia, dyspnea in 10% (n=7 each). The overall Grade 3/4 adverse events were thrombocytopenia 13% (n=9), neutropenia 12% (n=8), rash, fatigue 3% (n=2), pruritis, anemia, abdominal pain, diarrhea and myalgia 2% (n=1 each). No deaths occurred. *Summary/Conclusions.* Nilotinib has clinical activity and an acceptable safety and tolerability profile in patients with imatinib-resistant or intolerant CML in chronic phase.

1029

DELETIONS OF DERIVATIVE CHROMOSOME 9 IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKAEMIA: ONLY DELETIONS THAT SPAN THE ABL/BCR BREAKPOINT ARE ASSOCIATED WITH ADVERSE PROGNOSIS

S. Kreil,[1] K. Waghorn,[1] M. Müller,[2] A. Reiter,[2] R. Hehlmann,[2] A. Hochhaus,[2] N.C.P. Cross[1]

[1]Wessex Regional Genetics Laboratory, SALISBURY, United Kingdom; [2]III. Medizinische Klinik Mannheim, MANNHEIM, Germany

Deletions at the ABL-BCR reciprocal breakpoint on the derivative chromosome 9 are seen in 10-15% of patients with CML and have been associated with a poor prognosis, at least for cases treated with hydroxyurea or interferon α (IFN). Studies to date have used different FISH probe sets to determine deletion status and thus the results are not always directly comparable. Furthermore, information concerning the extent of deletions is limited. To provide more accurate information about deletion status, we have developed a rapid DNA-based screen based on multiplex ligation-dependent probe amplification (MLPA). Probes were designed to the deleted region both the upstream and downstream of the breakpoint, plus several control loci. MLPA was performed using standard conditions and peak heights were determined using an ABI 3100 Genetic Analyzer and Genotyper software. Since patient samples may contain a variable proportion of normal cells, we determined the sensitivity of the assay to detect the der(9) deletion in MC3 cells. We found that the deletion was readily detectable in dilutions of MC3 DNA in normal DNA at a level of 60% or greater, indicating that the assay was applicable to the great majority of CML patients. We then went on to perform a retrospective study of 348 patients (204 male; 144 female; median age 50 years, range 11-83) who had been enrolled into the German CML I, II or III studies between 1987 and 1999. This represents the largest study on the prognosis of der(9) deletions to date. All patients received IFN as first line therapy but 61 were subsequently treated with imatinib and 138 subsequently underwent stem cell transplantation (SCT). At the time of analysis, 161 patients were still alive at a median of 8.8 years (range 5.6-16.3). MLPA was performed on samples taken prior to treatment and showed that 59/348 (16.9%) of cases had der(9) deletions, defined as loss of at least two consecutive markers. Unexpectedly, we found that patients with deletions survived longer than those without deletions, although the difference was not significant (9.8 versus 7.3 years; p=0.17). This effect was seen both for cases that underwent SCT (not deleted: n=116, median = 9.4 years versus deleted: n=22, median not reached; p=0.34) or did not undergo SCT (not deleted: n=173, median = 6.8 years versus deleted: n=37, median = 9.8 years; p=0.27). However, the 21 cases who had deletions that spanned the translocation breakpoint did show inferior overall survival when compared to all other cases (5.7 versus 8.8 years; p=0.0025). This was not seen for patients with deletions on the ABL side only (n=20) or the BCR side only (n=18) and in fact both these groups showed longer survival compared to all other patients. In conclusion, MLPA is a reliable technique for detection of der(9) deletions in CML. Our analysis indicates that only those deletions that extend to both sides of the reciprocal ABL-BCR fusion breakpoint are associated with adverse prognosis.

Myelodysplastic syndromes

1030

EXPRESSION PROFILING OF CD34+ CELLS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES: INVOLVEMENT OF INTERFERON-STIMULATED GENES AND CORRELATION TO FAB SUBTYPE AND KARYOTYPE

A. Pellagatti,[1] M. Cazzola,[2] A. Giagounidis,[3] L. Malcovati,[2] M. Della Porta,[2] S. Killick,[4] L. Campbell,[1] L. Wang,[1] C. Langford,[5] C. Fidler,[1] D. Oscier,[4] C. Aul,[3] J. Wainscoat,[1] J. Boultwood[1]

[1]John Radcliffe Hospital, Oxford, United Kingdom; [2]IRCCS Policlinico S. Matteo, Pavia, Italy; [3]St Johannes Hospital, Duisburg, Germany; [4]Royal Bournemouth Hospital, Bournemouth, United Kingdom; [5]Sanger Institute, Hinxton, Cambridge, United Kingdom

The myelodysplastic syndromes (MDS) are a heterogeneous group of hematopoietic malignancies characterized by blood cytopenias, ineffective hematopoiesis and hypercellular bone marrow. We have used Affymetrix microarray technology to determine the gene expression profiles in CD34+ cells of MDS patients and controls. Fifty-five MDS patients (18 RA, 19 RARS and 18 RAEB) and 11 controls were included in the study. Twenty of 55 patients had a del(5q). CD34+ cells were isolated from bone marrow samples of patients and controls using MACS magnetic columns. Extracted total RNA was amplified using the Two-Cycle Target Labelling kit (Affymetrix). Biotin-labelled fragmented cRNA was hybridized to Affymetrix U133 Plus2.0 chips (47,000 transcripts, representing 39,000 human genes). Cell intensity calculation and scaling was performed using GeneChip Operating Software and data analysis using GeneSpring 7.2. The expression profiles of MDS $CD34^+$ cells showed many similarities to reported interferon-α induced gene expression in normal $CD34^+$ cells. Indeed the two most up-regulated genes, IFIT1 and IFITM1, are interferon stimulated genes (ISG). IFITM1 and IFIT1 were up-regulated by >2-fold in 37/55 MDS patients and by >10-fold in many cases. Genes down-regulated by >2-fold in the majority of MDS patients include the putative tumor suppressor gene Gravin/AKAP12, ARPP-21, CD24 and MME. Gravin/AKAP12 was down-regulated in 44/55 MDS patients. The results for several genes have been confirmed by real-time quantitative PCR (TaqMan). The association of distinct gene expression profiles with specific FAB and cytogenetic groups was determined. Hierarchical clustering performed using a set of 457 significantly different genes showed that MDS patients with RARS constitute a homogeneous group, while MDS patients with RA and RAEB show more overlap. $CD34^+$ cells from MDS patients with RARS showed up-regulation of mitochondrial-related genes, and in particular of those of heme synthesis (e.g. ALAS2). Statistical analysis showed that 889 probe-sets could discriminate MDS patients with a del(5q) from those without a del(5q). Approximately 40% of the 889 probe sets mapped to chromosome 5q and their expression levels were lower in MDS patients with del(5q) than those without del(5q), suggesting that the deletion in patients with a del(5q) has a gene dosage effect. MDS patients with the del(5q) showed distinctive up-regulation of the histone HIST1 gene cluster at chromosome 6p21 and of genes related to the actin cytoskeleton. In order to identify genes differentially expressed between early and advanced MDS, a comparison was made between the 18 patients with RA and the nine MDS patients with RAEBII. 762 significantly different probe sets were identified that could group together MDS patients with RAEBII. The most significant genes identified include CASP3 and FLT3, and represent potential prognostic markers or markers of disease progression. This study provides important and new insights into the pathophysiology of MDS.

Funding. This study has been funded by Leukaemia Research Fund.

1031

LENALIDOMIDE SELECTIVELY INHIBITS *IN VITRO* GROWTH OF THE MALIGNANT CLONE AND UP-REGULATES SPARC IN MYELODYSPLASTIC SYNDROME (MDS) PATIENTS WITH 5Q DELETION

B. Jädersten,[1] A. Pellagatti,[2] A.M. Forsblom,[1] E.K. Emanuelsson[1], M. Merup,[1] J. Samuelsson,[3] J.S. Wainscoat,[2] J. Boultwood,[2] E. Hellström-Lindberg[1]

[1]Karolinska Institutet, Stockholm, Sweden; [2]Leuk Res Fund Mol Haematology Unit, Oxford, United Kingdom; [3]South Hospital, Stockholm, Sweden

Backgrounds. In a phase II trial the immunomodulatory drug lenalidomide induced 75% complete cytogenetic remissions in patients with

myelodysplastic syndrome (MDS) and 5q31 deletion. Lenalidomide has been shown to inhibit angiogenesis, cell adhesion, and secretion of TNF-α, and to modulate other cytokines. Furthermore, lenalidomide stimulates T-cells and NK-cells, and directly induces apoptosis in myeloma cells. It is not well characterized how these effects are mediated, nor which effects that are central for the impact on cancer cells. *Aim*: To assess the direct effects of lenalidomide on growth, differentiation, and gene expression of hematopoietic cells from MDS patients with del(5)(q31) and healthy controls. *Methods.* Selected $CD34^+$ hematopoietic progenitors from 12 MDS patients with del(5)(q31) and from 10 healthy controls were cultured with or without 10 μM of lenalidomide in a 14-day model for erythroblast differentiation (with medium containing IL-3, IL-6, and SCF, with addition of Epo during the second week). FISH and FACS analyses were performed at day 0, 7, and 14. The median proportion of 5q- cells by FISH at day 7 was 98% (range 86-99), dropping to 84% (range 14-98) at day 14 due to a variable outgrowth of cytogenetically normal cells. Gene expression profiling was performed on day 7 cells from 6 MDS patients and 5 healthy controls using Affymetrix Human Genome U133 Plus 2.0 Arrays. *Results.* In erythroblast cultures with cells from healthy controls, lenalidomide had no inhibitory effect on fold increase of cell counts (p=0.60). However, in cultures with cells from 5q- patients, the clone with 5q deletion showed significant inhibition of fold increase at day 14 (p=0.04), while the cytogenetically normal progenitors were not inhibited (p=0.89). FACS analysis at day 14 showed that lenalidomide samples had higher proportions of cells expressing erythroid markers and lower proportions expressing myeloid markers. Gene expression profiling showed that four genes were up-regulated by lenalidomide in all MDS 5q- and control samples analyzed: *Z39IG*, *PPIC*, *TPBG* and *SPARC*. The median up-regulation of *SPARC* was 4.3-fold (range 2.3-9.4). *LRP11* and *HBA2* were down-regulated in 10 of 11 samples. Several of the differentially expressed genes warrant further investigation. The SPARC gene has been postulated to be a tumor suppressor gene in AML and has been shown to have anti-proliferative and anti-angiogenetic effects. Interestingly, the SPARC gene maps within the commonly deleted region (CDR) of the 5q- syndrome at 5q31-q32. *Conclusions.* Lenalidomide selectively inhibits in vitro growth of 5q- hematopoietic progenitors, while not affecting growth of cytogenetically normal cells from MDS patients with 5q deletion or from healthy controls. In addition, lenalidomide affects cell differentiation and induces changes in gene expression including up-regulation of SPARC. We hypothesize that one part of the potent effects of lenalidomide is mediated through increased SPARC expression. Whether the localization of the SPARC gene to the CDR of the 5q- syndrome is significant or not to the pathogenesis of the 5q- syndrome remains to be determined.

1032

CLINICAL BENEFIT FROM 2 PHASE II TRIALS EVALUATING LENALIDOMIDE (REVLIMID) IN LOWER-RISK MYELODYSPLASTIC SYNDROME PATIENTS WITH OR WITHOUT DEL 5Q CYTOGENETIC ABNORMALITIES

A.F. List,[1] G. Dewald,[2] J. Bennett,[3] A. Giagounadis,[4] A. Raza,[5] E. Feldman,[6] B. Powell,[7] P. Greenberg[8], D. Thomas[9], R. Stone[10], C. Reeder,[2] K. Wride[1],[1] J. Patin[1],[1] M. Schmidt[1],[1] J.B. Zeldis[11], R. Knight[11]

[1]*H. Lee Moffitt Cancer Center, Tampa, FL, USA;* [2]*Mayo Clinic, Rochester, MN, USA;* [3]*University of Rochester, Rochester, NY, USA;* [4]*St. Johannes Hospital, Duisberg, Germany;* [5]*University of Massachusetts, Worchester, MA, USA;* [6]*New York Cornell Medical Center, New York, NY, USA;* [7]*Wake Forest University, Winston Salem, NC, USA; 8Stanford University, Stanford, CA, USA; 9MD Anderson Cancer Center, Houston, TX, USA;* [1]*0Dana Farber Cancer Center, Boston, MA, USA;* [11]*Celgene Corporation, Summit, NJ, USA*

Transfusion-dependent myelodysplastic syndrome (MDS) is a serious illness which adversely impacts overall survival. Results from 2 phase II clinical trials have shown that oral lenalidomide produces meaningful hematological improvement in patients with low- or intermediate-1-risk MDS with or without an associated del 5q cytogenetic abnormality (*A. List et al EHA 2005, A. Raza et al. MDS 2005*). To investigate possible differences in efficacy and safety of lenalidomide in lower-risk MDS patients with or without an associated del 5q cytogenetic abnormality. Results from patients from 2 phase II clinical trials (MDS-002, MDS-003) of lenalidomide were analyzed. Differences in the frequency of red blood cell (RBC)-transfusion independence, improvement in hemoglobin, cytogenetic and pathologic response and safety were evaluated. In the del 5q patients, 67% (66/97) of the patients who had become RBC-transfusion independent remained RBC-transfusion independent. The duration of response was at least 24 weeks in 84% (83/97) of the responders and was at least 52 weeks in 53% (52/97) of the patients. The median increase in blood Hb from baseline to the maximum Hb achieved during RBC-transfusion independence was 5.5 g/dL (range, 1.1 11.4 g/dL, n = 99). Major cytogenetic responses were observed in 44% (32/72) and minor cytogenetic responses were observed in 29% (21/72) of the patients who were evaluable for cytogenetic response. Among patients with available follow-up bone marrow aspirate specimens, the follow-up bone marrow aspirates from 33% (27/81) of the patients had no evidence of MDS. In the non del 5q population, 26% (56/215) of the patients had achieved RBC-transfusion independence by ITT analysis during lenalidomide therapy. In this population, 77% had a normal karyotype and no differences were observed in transfusion independence rate between patients with a normal versus abnormal karyotype (29% and 27%, respectively). The duration of response was at least 24 weeks in 17% (36/215) of the responders and was at least 52 weeks in 10% (22/215) of the patients. Lenalidomide-induced transfusion independence was associated with a median increase from baseline in Hb of 3.0 g/dL in the responders. Neutropenia and thrombocytopenia were the most common adverse events and were reported at least once in 44% (172 and 174/395, respectively) of the patients who were treated with the 10 mg/day starting dose. Combined disease- and treatment-associated mortality (6%; 25/408) was relatively low and appeared consistent with the survival reported in the literature for lower-risk MDS. Lenalidomide is an effective and well-tolerated treatment for a select group of patients with low- or intermediate-1-risk MDS without an associated del 5q cytogenetic abnormality. From these data it becomes evident that there is a subpopulation of the non del 5q patients who respond similarly to lenalidomide compared with del 5q patients. Additional studies are warranted to investigate pathogenetic differences that account for the karyotype dependence in the frequency and durability of response.

1033

EFFECT OF LENALIDOMIDE (CC-5013) ON GENE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN MYELODYSPLASTIC SYNDROME WITH DEL(5Q) CHROMOSOME ABNORMALITY AND ITS RELEVANCE TO ANGIOGENESIS IN BONE MARROW

G. Buesche,[1] S. Dieck,[2] A. Giagounidis,[3] O. Bock,[2] L. Wilkens,[2] B. Schlegelberger,[2] R. Knight,[4] J. Bennett,[5] C. Aul,[3] H. Kreipe[2]

[1]*Medizinische Hochschule Hannover, Hannover, Germany;* [2]*MHH, Hannover, Germany;* [3]*St. Johannes Hospital, Duisburg, Germany;* [4]*Celgene Corporation, Warren, NY, USA;* [5]*University of Rochester Medical Center, Rochester, NY, USA*

Background. Lenalidomide, a novel immunomodulatory drug (IMiD), is a multifunctional inhibitor of angiogenesis. Vascular endothelial growth factor (VEGF) and its receptor (KDR) are major regulators of angiogenesis, which plays a key role in the growth and progression of solid tumors and hematologic neoplasms, and increased plasma levels of KDR were correlated with a lower remission rate in patients with myelodysplastic syndromes (MDS). *Methods*. From a total of 35 patients suffering from MDS with del(5q) and being treated within a multicenter trial on the efficacy of CC-5013, biopsies and aspirates from bone marrow taken before and 6 + 12 months after start of lenalidomide therapy were evaluated for the percentage of cells with del(5q), blast count, vascularization, and gene expression of VEGF and its receptor using cytologic, histopathologic, cytogenetic and molecular genetic methods. 20 patients with healthy marrow served as a control for normal marrow. *Results.* Before start of treatment, vascularity of bone marrow, measured as the total length of vessels within the marrow volume, was markedly increased in MDS exceeding that from normal marrow by a factor of 3.24 ± 2.57 (p<0.00005). Increase of vascularity correlated with increase of VEGF gene expression, which exceeded that from normal marrow by a factor of 2.68 ± 2.21 (P = 0.0003), whereas KDR gene expression was not significantly changed. During therapy with lenalidomide, vascularity markedly decreased to normal values in 60% of patients (21 / 35; p< 0.00005). Normalization of vascularization correlated with a major cytogenetic response of disease (< 35% metaphases with del(5q); p=0.0005) which occurred in 21 patients, 12 of them with a complete cytogenetic remission. Anti-angiogenic inefficacy of lenalidomide correlated with progression of disease (>= 5% blasts in blood or bone marrow; 14 patients; p=0.0005). In contrast to vascularity, VEGF and KDR gene expression were not reduced during lenalidomide treatment; the correlation between vascularity and VEGF gene expression disappeared (p< 0.00005). *Conclusions.* In MDS with del(5q), lenalidomide uncouples angiogenesis from the effect of VEGF resulting in a significant reduction of marrow vascularity followed by an increase in gene expression of

VEGF and KDR. Inhibition of angiogenesis correlates with a significant reduction of the MDS clone in bone marrow whereas increase of vascularization indicates progression of disease.

1034

AN ABERRANT MRNA SPLICING PHENOTYPE IN MYELODYSPLASTIC SYNDROMES (MDS)

P. Steensma,[1] J. Caudill,[1] J.C. Porcher,[1] M.E. Nelson,[2] S.H. Kaufmann[1]

[1]*Mayo Clinic, Rochester MN, USA;* [2]*University of South Dakota, Vermillion, SD, USA*

Most mammalian genes undergo alternative pre-mRNA splicing, which generates diverse transcripts and protein isoforms that may be regulated in a tissue- or developmental stage-specific manner. Aberrant and alternative splicing may be associated with neoplasia, and can contribute to alterations in gene expression detectable by oligonucleotide microarrays, though the function of the novel spliceoforms in cancer pathogenesis is, for the most part, unknown. While studying *ATRX*, an X-linked gene encoding a chromatin-associated transcriptional regulator that was recently shown to be mutated in patients with MDS and an acquired thalassemic phenotype (*Gibbons RJ et al. Nature Genetics 2003 and Steensma DP et al. Blood 2004*), we discovered a novel exon-skipping and frameshifting alternative spliceoform in the region of the gene that encodes the conserved helicase domain of the protein. A *cis*-acting genomic DNA mutation of *ATRX* was not detected, leading us to hypothesize that aberrant splicing as a consequence of trans-acting defects altering basal splicing machinery or regulators of alternative splicing might be common in MDS. To further characterize aberrant/alternative splicing of *ATRX* and other representative genes in MDS. We performed RT-PCR of marrow and blood cells from patients and healthy controls of *ATRX*, CDC25C (a gene at 5q31.1 that encodes a phosphatase important in cell cycle regulation, with expression that changes during lenalidomide therapy), and *HELLS/LSH/PASG/SMARCA6* (encodes a SWI/SNF2-related helicase that, like *ATRX*, localized to pericentromeric heterochromatin). Amplicons were analyzed by DNA sizing column chromatography under non-denaturing conditions, cloned into DH5α competent cells using the pGEM-T Easy system, and sequenced. The novel aberrant *ATRX* exon-skipping transcript was not present in 20 varieties of normal tissue from autopsies (gut epithelium, testis, myocardium, etc.), and was detected in blood cells from only 1 of 24 healthy volunteers (transiently). In contrast 7/13 patients with MDS and 4/16 patients with myeloid leukemia exhibited the *ATRX* variant in hematopoietic cells, in some patients in equal or greater proportion than the normal transcript. In MDS patients treated with chemotherapy who achieved a cytogenetic remission, the aberrant *ATRX* transcript disappeared, and was again detectable at the time of disease relapse. We also observed a series of novel exon-skipping or intron-retaining alternative spliceoforms of CDC25C that were not present in healthy controls, and splicing patterns of *HELLS/LSH/PASG/SMARCA6* were likewise disrupted in MDS primary samples. A subset of patients with MDS may exhibit a generalized defect in pre-mRNA splicing that leads to the generation of aberrantly spliced isoforms in multiple genes of potential pathobiologic relevance. The etiology and functional significance of these variants should be explored further. Because the nature of neoplasia-associated alternative gene products is often consistent with an active role in cancer, this suggests a potential therapeutic target in malignancies such as MDS that display aberrant splicing.

Antibodies in the treatment of CLL

1035

AUTOLOGOUS GRAFT VERSUS HOST DISEASE (AUTO-GVHD) AFTER AN ALEMTUZUMAB CONTAINING CONDITIONING REGIMEN AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN CLL: IMMUNOLOGICAL MECHANISMS AND POTENTIAL ANTI-LEUKEMIA EFFECT

T. Zenz,[1] M. Ritgen,[2] A. Mackensen,[3] P. Dreger,[4] A. Kröber[1], T. Barth,[1] R. Schlenk,[1] S. Böttcher,[5] M. Hallek,[6] M. Kneba,[5] H. Döhner,[1] S. Stilgenbauer[1]

[1]*University of Ulm, Ulm, Germany;* [2]*University of Kiel, Kiel, Germany;* [3]*University of Regensburg, Regensburg, Germany;* [4]*University of Heidelberg, Heidelberg, Germany;* [5]*University of Schleswig-Holstein, Kiel, Germany;* [6]*University of Cologne, Cologne, Germany*

A high incidence of unexplained skin rashes and auto-GvHD was observed after an alemtuzumab containing myeloablative conditioning regimen and autologous stem cell transplantation (SCT) in patients with CLL. 1) Comparison of CLL patients undergoing autologous stem cell transplantation after conditioning with TBI/Cy ± Alemtuzumab. 2) Detailed analysis of the defects of immune reconstitution. 3) Analysis of the influence of auto-GvHD on minimal residual disease. Methods. We treated 27 patients with CLL (Binet B / C) with autologous SCT in two trials of the German CLL Study Group at the University of Ulm (CLL3 & CLL3C trials). Patients received cytoreduction with fludarabine plus cyclophosphamide and stem cell mobilization with Dexa-BEAM. In the CLL3 trial (n=11) autologous SCT was performed after standard conditioning with 12 Gy total body irradiation and cyclophosphamide (120 mg/kg) (TBI/Cy). Patients in the CLL3C trial (n=16) were treated identically except for the addition of alemtuzumab before SCT (mean 100 mg iv) (Alem/TBI/Cy). There were no skin rashes or auto-GvHD in the standard TBI/Cy group. In contrast, 12 of 16 patients (75%) receiving Alem/TBI/Cy developed a skin rash (maculopapular rash (n=5), erythrodermia (n=3), eczema (3)) between 43 and 601 days after SCT. In 7 patients a clinical diagnosis of auto-GvHD was made. Typically, concurrent symptoms at the onset of auto-GvHD included conjunctivitis (n=4), sicca syndrome (n=5), and cholestasis (n=4). The histological findings were compatible with GvHD grade 1-2 in all five patients with clinical auto-GvHD in whom skin biopsies were performed. The median duration of GvHD was 517 days (range 60-867) and the reduction of immunosuppression led to a flare of the skin rash in 5 of 7 patients. The reconstitution of CD4 and CD8 positive cells was severely delayed in the Alem/TBI/Cy group with a particular depletion of CD8+ cells for up to 2 years. The CD4/CD8 ratio was abnormally high in the Alem/TBI/Cy group. This increased ratio was mainly caused by the extreme CD4/8 ratio imbalance in patients with GvHD. The CD4/8 ratio was 20 times higher among patients with auto-GvHD as compared to patients without GvHD (all time points combined). Interestingly, histology showed a predominant invasion of the skin by CD4 positive T lymphocytes. Molecular analysis revealed oligoclonal expansion of T cells in the skin biopsy samples of patients with GvHD with similar Vβ subfamilies (BV3, BV18)(n=3). The addition of alemtuzumab led to continuous MRD negativity in 10/14 patients (71%) compared to 0/7 patients receiving TBI/Cy (p=0.0039). Within the Alem/TBI/Cy group continuous MRD negativity was observed in 6/6 patients with auto-GvHD (100%) vs. 4/8 patients without auto-GvHD (50%; p=0.08) . The current study demonstrates a remarkable incidence of a GvHD-like syndrome attributable to the addition of alemtuzumab to the TBI/Cy conditioning regimen before autologous SCT in patients with CLL. The addition of alemtuzumab to the conditioning regimen led to improved disease control at the molecular level and longer follow-up will show if the GvHD-like syndrome will lead to an anti-leukaemia effect and prolonged MRD negativity.

1036

PRELIMINARY RESULTS FOR THE PHASE III TRIAL OF ALEMTUZUMAB (CAMPATH) VS CHLORAMBUCIL AS FIRST-LINE TREATMENT FOR B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

P.H. Hillmen,[1] A.S. Skotnicki,[2] T.R. Robak,[3] B.J. Jaksic,[4] A.D. Dmoszynska,[5] C.S. Sirard,[6] J.M. Mayer[7]

[1]*The General Infirmary at Leeds, Leeds, United Kingdom;* [2]*Jagiellionian University Collegium Medic, KRAKOW, Poland;* [3]*Kopernik Memorial Hospital, Lodz, Poland;* [4]*Clinical Hospital Merkur, Zagreb, Croatia;* [5]*Clinical Hospital No. [1], Lublin, Poland;* [6]*Genzyme, Boston, USA;* [7]*University Hospital Brno, Brno, Czech Republic*

Chlorambucil (CHLO) is an approved therapy for patients with B-CLL. Alemtuzumab (CAM) has suggested effectiveness in untreated and demonstrated efficacy in relapsed and refractory B-CLL. CAM307 is an international, randomized, open-label study comparing efficacy and safety of CAM versus CHLO in previously untreated patients with B-CLL. Presented are the preliminary safety and efficacy results of this study. Eligible pts were Rai stage I-IV with evidence of progression requiring therapy. Patients with secondary malignancies, autoimmune thrombocytopenia, active infection, central nervous system involvement, or who were positive for cytomegalovirus (CMV) via quantitative PCR, were excluded from the trial. Patients were randomized 1:1 to receive either CAM 30 mg IV 3 times a week for up to 12 weeks or CHLO 40 mg/m^2 PO on day one of a 28-day cycle for up to 12 cycles. All patients in the CAM arm received prophylaxis with trimethoprim/sulfamethoxazole DS and famciclovir during therapy and until CD4$^+$ counts returned to ≥200 cells/μL. Accrual completed with 297 patients (213 males, 84 females; median age 60 yrs [range: 35-86]); CAM n=149 and CHLO n=148. Treatment arms were well balanced for key prognostic factors analyzed to date; overall, 96% were WHO PS 0-1, 70% had < 5 cm lymphadenopathy. Median length of treatment was 11.7 wks for CAM and 24.4 wks for CHLO. An independent analysis of response showed an 82.6% Overall Response (OR) in the CAM arm compared to 54.7% OR in CHLO (p<0.0001), and patients in the CAM arm had a significantly higher CR rate compared to those in the CHLO arm: 22.1% vs 2.0% (p<0.0001). Preliminary analysis of the pertinent safety data reported through October 24, 2005 is summarized in the Table.

Table 1. Phase III CAM study.

Safety Grade 3/4 events	*CAM*	*CHLO*	*p value**
Thrombocytopenia	17.7%	15.6%	0.7546
Anemia	12.2%	15.0%	0.6103
Neutropenia	42.2%	23.1%	0.0007
Febrile neutropenia	4.8%	3.4%	0.7697
Infection (excluding CMV)	14.3%	6.8%	0.0562
CMV infection	6.8%	0.0%	0.0017

**Comparisons were made using the Exact method.*

Overall safety data indicate 34.7% of CAM patients and 19.7% of CHLO patients experienced a serious adverse event, with 21.1% and 4.1% considered drug related, respectively. One treatment related death occurred in the CHLO arm and none in the CAM arm. Infusion related events (fever, rigors, nausea, vomiting, and hypotension) were the most frequently reported CAM related events; however the severity was predominately grade 1/2 whereby only 13.6% of patients experienced a grade 3/4 event. The only pertinent grade 3/4 safety signals which attained statistical significance were neutropenia and CMV infection. As expected, the incidence of infection in the CAM arm was higher than the CHLO arm. However, there was no difference in the incidence of febrile neutropenia in the two study arms. Preliminary analysis of this randomized controlled trial shows that CAM had an OR and CR rate statistically superior to CHLO with manageable toxicity.

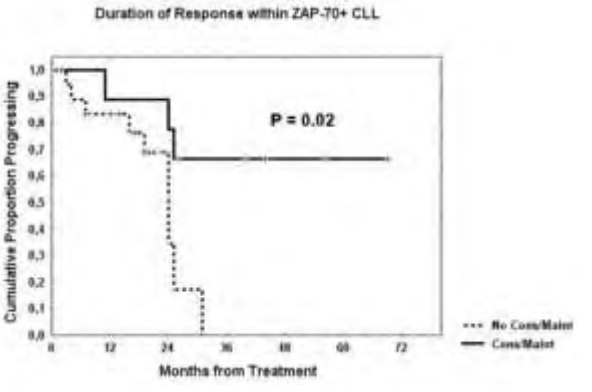
1037

CONSOLIDATION AND MAINTENANCE THERAPY WITH RITUXIMAB PROLONG DURATION OF RESPONSE BOTH WITHIN ZAP-70 POSITIVE AND NEGATIVE CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

G. Del Poeta,[1] M.I. Del Principe,[1] P. Niscola,[1] L. Maurillo[1], F. Buccisano,[1] A. Venditti,[1] C. Mazzone,[1] R. Marini,[1] A. Zucchetto,[2] G. Suppo,[1] F. Luciano,[1] V. Gattei,[2] F. Lo Coco,[1] P. De Fabritiis[1], S. Amadori[1]

[1]*Hematology, University Tor Vergata, Rome, Italy;* [2]*Hematology Unit, CRO, IRCCS, Aviano (PN), Italy*

Clinical trials of monoclonal antibodies in combination with chemotherapy have reported improved outcome in CLL because this approach reduces disease burden to levels detectable only by flow cytometry or molecular methods. Along this line, we have recently published that rituximab in sequential combination with fludarabine (Flu) for symptomatic, untreated CLL allowed us to achieve higher remission rates and longer duration of response (Cancer, 2005). Recent literature data indicate that unmutated VH genes, CD38 and/or ZAP-70 protein tyrosine kinase overexpression may predict a worse outcome. We performed a phase II study that added rituximab sequentially to Flu as initial therapy for symptomatic, untreated CLL in order to evaluate both the clinical response and outcome. In about one third of the patients we added a consolidation /maintenance therapy with rituximab in order to prolong even more the response duration. ZAP-70 protein was determined before chemotherapy on mononuclear cells by flow cytometry using anti-ZAP-70 Alexa Fluor 488 (Caltag Laboratories) conjugated antibody. Seventy-two CLL patients, median age 60 years (range 37-74) received six monthly courses of Flu (25 mg/sqm for 5 days) and four weekly doses of rituximab (375 mg/sqm) starting on an average of thirty days (range 21-150) after completion of Flu therapy. According to modified Rai stages, 4 patients had a low stage, 67 an intermediate stage and 1 a high stage. Based on NCI criteria, 56/70 (80%) patients achieved a complete remission (CR), 12/70 (17%) a partial remission (PR) and 2/70 (3%) a stable disease (SD). Three patients presented grade 3 (WHO) infective lung toxicity and 1 patient acute fatal B hepatitis. Hematologic toxicity included mainly neutropenia (grade 3 and/or 4 in 37 pts) and thrombocytopenia (grade 3 and/or 4 in 4 pts). Twenty six patients, either with CD5$^+$CD19$^+$ bone marrow cells >1% (n=16 pts) or presenting CD19$^+$CD5$^+$ peripheral lymphocytes >1000/μL (n=10 pts) within six months after completion of the induction treatment, underwent consolidation/maintenance therapy with four monthly cycles of rituximab at 375 mg/sqm followed by eight/twelve monthly doses of rituximab at 150 mg/sqm. The median follow-up duration was 36 months. Noteworthy, all B-CLL pts experienced a very long progression-free survival (PFS) from treatment (71% at 5 years). Nevertheless, CLL patients that underwent consolidation therapy showed a significant longer duration of response (87% vs 54% at 5 years, p=0.02). ZAP-70 was positive (>20%) in 35/72 (49%) pts and a significant shorter PFS was observed in ZAP-70+ pts (36% vs 95% at 5 years; p=0.0002). Noteworthy, within the consolidated patients subset (n=26), ZAP-70$^+$ pts (n= 11) showed a worse PFS (67% vs 100% at 5 years, p=0.02). However, interestingly, within the ZAP-70$^+$ subset (n=35), the consolidated patients (n=11) showed a significant longer duration of response (67% vs 0% at 2.5 years, p=0.02, Figure) in comparison with non consolidated patients (n=24). Therefore, the addition of consolidation/maintenance therapy with rituximab significantly prolongs duration of response allowing a better outcome. Finally, this immunotherapeutic supplement seems to improve significantly the clinical outcome of pts notoriously with a bad prognosis, such as ZAP-70+ B-CLL.



1038

LUMILIXIMAB IN COMBINATION WITH FLUDARABINE, CYCLOPHOSPHAMIDE, AND RITUXIMAB (FCR) FOR PATIENTS WITH RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

C. Byrd,[1] T. Kipps,[2] S. O'Brien,[3] I. Flinn,[4] A. Forero,[5] D. Wynne,[6] A. Molina[6]

[1]*The Ohio State University, Columbus, OH, USA;* [2]*University of California, San Diego, San Diego, CA, USA;* [3]*MD Anderson Cancer Center, Houston, TX, USA;* [4]*Johns Hopkins University, Baltimore, MD, USA;* [5]*University of Alabama at Birmingham, BIRMINGHAM, AL, USA;* [6]*Biogen Idec, San Diego, CA, USA*

Background. A recently completed phase 1, single-agent study with lumiliximab showed evidence of clinical activity and a favorable safety profile. Preclinical data suggesting synergy with both fludarabine and rituximab resulted in initiation of a combination study of lumiliximab with FCR in previously treated patients with CLL. *Aims*. The objectives of this study are to determine the safety profile of lumiliximab in combination with FCR, recommend a phase 2 dose, and evaluate the clinical activity of lumiliximab in combination with FCR. *Methods*. Patients 18 years of age or older with relapsed $CD23^+$ B-cell CLL were eligible for this open-label, dose-escalation, phase 1/2 study. For the phase 1 portion of the study, patients received either 375 mg/m^2 or 500 mg/m^2 of lumiliximab in combination with each 28-day cycle of FCR for 6 cycles. *Results.* No dose-limiting toxicity was noted in the phase 1 portion of this study (375 mg/m^2 dose, n=3, and 500 mg/m^2 dose, n=6) and 500 mg/m^2 was the recommended phase 2 dose. In total, 31 patients were enrolled between June 2004 and January 2006: 3 at 375 mg/m^2 and 28 at 500 mg/m^2. Data on the first 20 patients are presented. Patients had progressive, symptomatic CLL as defined by NCI criteria, median of 2 prior treatments (range, 1 to 9), median age of 57, 60% were males, 95% had WHO performance status of 1. Fourteen patients experienced CTC Grade 3 or 4 adverse events. Events reported in more than 1 patient were neutropenia (7 patients), leukopenia (4 patients), febrile neutropenia (3 patients), thrombocytopenia (2 patients), and pyrexia (2 patients). Hematologic toxicity is comparable with the FCR regimen. Sixteen patients completed ≥3 cycles of treatment and 4 patients completed ≤2 cycles. Response was evaluated using NCI-WG criteria. Nine (45%) patients have confirmed complete responses, 5 (25%) have partial responses, and 6 (30%) patients have disease progression. *Summary/conclusions*. Lumiliximab in combination with FCR is well tolerated and has shown no increased toxicity compared with FCR alone. Lumiliximab may enhance the CR rate with FCR when used for treatment of patients with progressive CLL after prior therapy.

1039

INTERIM REPORT OF THE UKCLL02 TRIAL: A PHASE II STUDY OF SUBCUTANEOUS ALEMTUZUMAB PLUS FLUDARABINE IN PATIENTS WITH FLUDARABINE REFRACTORY CLL (ON BEHALF OF THE NCRI CLL TRIALS SUB-GROUP)

H. Sayala,[1] P. Moreton,[2] R. Jones,[1] A.C. Rawstron,[1] S.J. O'Connor,[1] P. Evans,[1] A. Carter,[3] C. Dearden,[4] E. Matutes,[4] A.R. Pettitt,[3] B.D. Kennedy,[5] P. Moreton,[2] P. Hillmen[1]

[1]*HMDS, LEEDS, United Kingdom;* [2]*Pinderfields General Hospital, WAKEFIELD, United Kingdom;* [3]*Royal Liverpool University Hospital, LIVERPOOL, United Kingdom;* [4]*Institute of Cancer Research, LONDON, United Kingdom; Leicester Royal Infirmary, LEICESTER, United Kingdom*

Patients with fludarabine refractory CLL have a median survival of 10 months with conventional chemotherapy. Intravenous (IV) alemtuzumab is approved in fludarabine refractory CLL resulting in 33 to 50% responses. Combined alemtuzumab and fludarabine can induce responses in CLL refractory to both agents. Infusion reactions and 2-hour infusions 3x a week for 12 weeks are problems with IV alemtuzumab. Subcutaneous (SC) alemtuzumab is more convenient but pharmacokinetics suggest the need for prolonged therapy with little efficacy data in fludarabine-refractory CLL. A study to assess the safety and effectiveness of SC alemtuzumab in fludarabine-refractory CLL.Methods SC alemtuzumab was given at a dose of 30 mg 3x a week (after dose escalation) for up to 24 weeks depending on 6-weekly marrow assessments. Patients failing to respond to alemtuzumab in the trial could receive oral fludarabine (40 mg/m^2/day for 3 days every 4 weeks) combined with SC alemtuzumab. In this planned interim analysis of the first 44 patients (median age 66, range 41 to 79) 2 patients died before receiving alemtuzumab, and 5 remain on therapy. Of the remaining 37 patients, one withdrew consent and 36 patients have completed therapy. Responses to alemtuzumab monotherapy (n=36) were 2 MRD negative CR, 1 MRD positive CR, 11 PR (including 1 MRD negative patient who remained cytopenic), 20 NR and 2 died. Alemtuzumab was given for a median 12 weeks (range: 2-24) with a median dose of 913 mg (range 106 to 2173mg). 12 patients (8 NR and 4 PR) received concurrent fludarabine and SC alemtuzumab (median 2.5 courses fludarabine [range 1-3]). Two non-responders achieved a PR and one of the partial reponders achieved a CR (MRD positive). Therefore the overall response rate for the whole cohort was 16/36 (44%) including 3 MRD negative patients (2 CRs and 1 PR). IgVH gene was unmutated (>98% homology to germ line DNA) in 11/14. FISH revealed poor risk deletions (11q and/or 17p) in 21/34 patients (17p- in 9; 11q- in 6 and both in 6). p53 functional analysis is available for 23. 20/23 had p53/ATM dysfunction or deletion. 13/25 (52%) of ptients with poor risk deletions(11q and/or 17p) or p53 dysfunction responded to therapy. The initial alemtuzumab dose was associated with localised erythematous skin reactions in 20 patients (diameter 1 to 18 cm), fever in 7 and rigors in 3. All reactions subsided in <48h. Serious infections during alemtuzumab monotherapy were: CMV reactivation (10); febrile neutropenia (9); invasive fungal infection (3); pneumonia (2). On the combination, CMV reactivation in 2 cases but no other grade 3+ infections. All CMV reactivations resolved on antiviral therapy. Grade 3+ thrombocytopenia and neutropenia was seen in 16 and 25 patients on alemtuzumab monotherapy as well as in 1 and 2 patients on combined therapy, respectively. We report that subcutaneous alemtuzumab is effective in poor-risk fludarabine-refractory CLL and is well tolerated compared to IV therapy. A longer duration of SC alemtuzumab therapy (up to 24 weeks) is required. The addition of oral fludarabine improves the response rates with acceptable toxicity.

Clinical studies in Non-Hodgkin's Lymphoma

1040

SURVIVAL IMPACT OF THE TIME REPEATED BCL2/IGH REARRANGEMENT MEASUREMENTS IN FOLLICULAR LYMPHOMA PATIENTS TREATED WITH FRONT-LINE AUTO-TRANSPLANTATION IN THE GELF-94 TRIAL BY THE GELA

F. Broussais,[1] N. Mounier,[2] C. Sebban,[3] P. Brice,[4] E. MacIntyre,[5] P. Brice,[4] M.H. Delfau-LaRue,[6] J.M. Cayuela,[4] N. Brousse,[7] C. Haioun,[6] P. Feugier[8], H. Tilly[9], B. Coiffier[10], G. Salles[11]

[1]*Centre Hospitalier Lyon-Sud, Pierre-Benite, France;* [2]*Hopital Saint-Louis, Paris, France;* [3]*CLB, Lyon, France;* [4]*Saint-Louis, Paris, France;* [5]*Necker, Paris, France;* [6]*Henri Mondor, Paris, France;* [7]*APHP, Paris, France;* [8]*Aphp, Paris, France;* [9]*CHU, ROUEN, France;* [10]*CHLS, Pierre-Benite, France;* [11]*Lyon-Sud, Pierre-Benite, France*

Backgrounds. This study was undertaken to assess the impact of molecular residual disease (MRD) on survival controlling for consolidation treatment of two regimens: an autotransplantation framework or chemotherapy with interferon, in the treatment of high burden follicular lymphoma. MRD was defined as disappearance of Bcl2-IgH amplification by PCR. *Aims.* From 07/94 to 03/01, we have performed a prospective study, the GELF-94 trial, which randomized consolidation treatment after achieving clinical response between front line ASCT and chemotherapy. Of 401 patients included, 209 received 12 cycles of CHVP (cyclosphosphamide, doxorubicin, vincristine and prednisone) plus interferon α in 18 months (CHVP-I arm) and 192 received 4 cycles of CHOP (cyclosphosphamide, doxorubicin, vincristine and prednisone) then high-dose therapy with total body irradiation and ASCT (CHOP-HDT arm). *Methods.* Bone marrow (BM) and peripheral blood (PB) samples were obtained prospectively at diagnosis and repeated every 6 months during the first year then annually for PCR analysis in 12 laboratories. A standard PCR technique with one step of amplification was used for MBR and mcr breakpoints. Time repeated measurement was stopped at clinical relapse or instigation of a new treatment. At diagnosis, 225 patients had material available for Bcl2/Igh rearrangement analysis: BM for 182 (45%) patients and blood for 199 (50%). In the latter, 105 of the 199 patients (53%) were found to have a bcl2/IgH rearrangement in MBR breakpoint, whereas bcl2/IgH rearrangement in mcr was observed in 5 patients (3%) and no rearrangement at MBR or mcr in the remaining 94 patients (44%). No differences were found according to bcl2/IgH rearrangement in terms of response rate (82% for bcl2- vs. 80% for bcl2+) and 5 years survival (85% for bcl2- and 79% for bcl2+). Time repeated Bcl2/IgH rearrangement measurements were available for 142 patients (ASCT n=75, chemo n=67): in BM for 79 patients and in blood for 85 patients. There was no statistically significant difference in clinical characteristics between patients with/without time repeated measurement. *Results.* At a median follow-up of 64 months, the significant prognostic factors for survival were age below 40 yrs (RR= 21, p=0,005), complete clinical response (RR=5, p=0.02) and bcl2/IgH rearrangement negativity (RR=4, p=0.03), by time dependant Cox's model. There was no treatment impact. These findings confirm the importance of molecular response in addition to the clinical response as a critical factor for prognosis. *Conclusion*: No matter whether after chemotherapy alone of after autologous bone marrow transplant, patients in complete clinical and molecular remission show a significantly longer overall survival.

1041

LONG TERM FOLLOW UP OF 566 PATIENT WITH FOLLICULAR LYMPHOMA INCLUDED FROM 1986 TO 1995 IN THE GELF86 STUDY BEFORE RITUXIMAB AREA

P. Brice,[1] G Salles,[2] C. Haioun,[3] H. Tilly,[4] S. Le Gouill,[5] C. Foussard,[6] C. Sebban,[7] J. Gabarre[8], P. Solal-Celigny[9]

[1]*Hopital Saint Louis, Paris CEDEX 10, France;* [2]*CH Lyon Sud, Lyon, France;* [3]*Hopital Henri Mondor, Creteil, France;* [4]*Centre Henri Becquerel, Rouen, France;* [5]*Chu Hotel Dieu, Nantes, France;* [6]*CHU Angers, Angers, France;* [7]*Centre Leon Berard, Lyon, France; 8Hopital de la Piti, Paris CEDEX 10, France; 9Centre Jean Bernard, Le Mans, France*

Since 1998, rituximab has been authorized in FL and had shown survival benefit in recent studies. We wanted to analyze our previous protocol with a long term follow-up to estimate survival before the regular use of rituximab. The GELF 86 protocol was an open phase 3 trial initiated on 566 untreated FL patients coming from 40 participating medical centers and included from 1986 to 1995. At study entry, patients were classified in two groups according to the presence (high tumor burden HTB) or not (low tumor burden LTB) of one of the following parameters: nodal or extranodal tumor mass with a diameter over 7 cm; involvement of three nodal sites (over 3 cm); B-symptoms; large splenomegaly; serous effusion; local risk of compression and leukemia or blood cytopenia Patients with a LTB were randomly assigned to: no treatment until progression, prednimustine for 18 months or interferon α-2b for 18 months. Patients with a high HTB were randomly assigned to: CHVP (cyclophosphamide 600 mg/m^2, doxorubicin 25 mg/m^2, teniposide 60 mg/m^2 and prednisone 40 mg/m^2 × 5 days) monthly for 6 months then, after response, every two months for 12 months; CHVP plus interferon α-2b (5 MU/day 3 times per week) for 18 months.

Table.

	Characterisitics of patients	
	Low TB	*High TB*
Median age	52 y	52.5y
Sex ratio M/F	1.2	1.2
Abnormal LDH	10%	27%
Stage IV	60%	78%
BM involvement	56	60%
Median survival	11 y	7.5y

Initial prognostic factors were validated with 4.5 years longer median survival in patients with LTB but no plateau in both groups. For patients with LTB, the long term survival was similar according to the three randomization arms confirming that waiting for treatment until symptoms did not have any impact on prognosis. For patients with HTB, the benefit of adding interferon to a doxorubicin containing chemotherapy was confirmed (median survival 5.6 versus 7.6 years, p= 0.01) With a 10 year follow-up 55% of patients are alive in the LTB group and 40% in the HTB, and 89 patients are still in first remission At first relapse 239 patients had documented progression and 55 histological transformation were observed (similar rates according to initial tumor burden). Conclusion, despite a long median survival in patients with FL, no plateau has yet been reached but some patients experienced a very long survival.

1042

GAINS ON CHROMOSOME BAND 18Q21 PREDICT POOR OUTCOME IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA: RESULTS FROM A COMPARATIVE GENOMIC HYBRIDIZATION ANALYSIS WITHIN A MULTICENTRIC TRIAL (NHL-B-TRIAL)

A. Viardot,[1] A.C. Feller,[2] M. Kloess,[3] M. Löffler,[3] H.W. Bernd,[2] G. Ott,[4] S. Weendorf,[5] C. Schwaenen,[5] M. Baudis,[6] P. Lichter,[7] M. Pfreundschuh[8], H. Döhner,[5] L. Trümper[9], M. Bentz[10]

[1]*Universitätsklinikum Ulm, Ulm, Germany;* [2]*Institut fr Pathologie, Lbeck, Germany;* [3]*Institut fr Medizinische Informatik, Leipzig, Germany;* [4]*Pathologisches Institut, Wurzburg, Germany;* [5]*Abteilung Innere Medizin III, Ulm, Germany;* [6]*Division of Pediatric Hematology, Gainesville, USA;* [7]*Abteilung Molekulare Genetik, DKFZ, Heidelberg, Germany; 8Innere Medizinische Klinik I, Homburg, Germany; 9Abteilung Hmatologie und Onkologie, Gottingen, Germany;* [10]*Medizinische Klinik II, Karlsruhe, Germany*

Backgrounds. In Non-Hodgkin lymphomas, there are only few data regarding the prognostic significance of specific genomic aberrations. No analyses correlating genomic aberrations with the clinical course have been performed in homogenous cohorts of patients treated within a clinical trial. *Aims/Methods.* We used comparative genomic hybridisation (CGH) to perform such an analysis in diffuse large B-cell lymphomas (DLBCL). 367 paraffin-embedded tumor samples were obtained from patients, who where treated within the NHL-B trial of the German High-Grade Non-Hodgkin's Lymphoma Study Group. In this trial, all patients received similar therapy regimens (CHOP or CHOEP administered every 14 or 21 days). *Results.* CGH analysis was successful in 256 out of 367 cases (70%). 186 patients out of this series had a histology of DLBCL according to the WHO classification. In 137 of 186 cases (74%), imbalanced chromosomal aberrations were found (range from 1 to 25; median 4.5). The most frequent chromosomal changes (>15% of all cases) were gains involving chromosome bands 1q21 (16%), 7p21 (15%), 7q11 (16%), 17q22 (16%), 18q21 (22%) as well as losses on 4q31 (15%), 6q21 (23%) and 13q21 (17%). 43 high-level DNA amplifications were found in 26 cases, most frequently involving the chromosomal bands

18q21 (14 cases) and 2p13 (5 cases). Median follow-up time was 67 months. In an univariate analysis, gains on chomosome 3 (3p14: p<0.001, overall survival (OS), p=0.001, time to treatment failure (TTF); 3q22: p<0.001, OS, p=0.002, TTF; 3q27: p<0.001, OS, p<0.001, TTF), on chromosome arm 12p12 (p=0.005, OS, p=0.06, TTF) and on chromosome arm 18q21 (p<0.001, OS; p=0.002, TTF), as well as losses on 17p13 (p=0.09, OS; p<0.001, TTF) were associated with an inferior prognosis. In a multivariate model including the clinical parameters of the International Prognostic Index (IPI), 18q gain remained as an independent negative prognostic marker (OS: relative risk: 2.1 (1.3-3.5), p=0.004; TTF: relative risk: 1.9 (1.2-3.0) p=0.006). Subset analysis revealed that 18q gains are particularly predictive in the IPI-low and intermediate-low group (p<0.001). In 120 cases, data about bcl-2 expression were available. The negative prognostic impact of bcl-2 expression for overall survival was restricted to cases with an additional 18q gain (p=0.02). *Summary*: These data demonstrate that molecular cytogenetic studies can be performed in the context of a clinical lymphoma trial. Genomic changes can improve the risk assessment in DLBCL.

1043

ASSESSMENT OF DISEASE DISSEMINATION IN GASTRIC VERSUS EXTRAGASTRIC MALT LYMPHOMA USING EXTENSIVE STAGING: A SINGLE CENTER EXPERIENCE OF 140 PATIENTS

M. Raderer,[1] S. Woehrer,[1] B. Streubel,[1] M. Troch,[2] K. Turetschek,[1] U. Jäger,[1] C. Skrabs,[1] A. Gaiger,[1] J. Drach,[1] A. Puespoek[1], M. Formanek,[1] M. Hoffmann,[1] W. Hauff,[1] A. Chott[1]

[1]*University of Wien, Wien, Austria;* [2]*Medical University of Wien, Wien, Austria*

Backgrounds. Molecular data as well as preliminary clinical findings have suggested MALT lymphoma as a multifocal disease in a high percentage of patients. We report our findings with an extensive staging routine applied in patients diagnosed with MALT lymphoma at our institution. *Patients and Methods.* A total of 140 consecutive patients underwent staging according to a standardized protocol. Sixty-one had gastric lymphoma, while 79 had been diagnosed with extragastric MALT lymphoma. The majority of these latter patients had salivary gland lymphoma (n=24, 30%, with 22 parotid and 2 submandibular gland lymphomas), 17 had lymphoma of the orbit/lacrimal gland (20%), another 11 had MALT lymphoma originating in the lung (14%) and 10 had primary intestinal MALT lymphoma (12.5%, 8 in the colorectum and two in the small intestine). The remaining patients suffered from lymphoma of the thyroid (n = 5), conjunctiva (n = 4), the breast (n=3), the liver (n=2) and the kidney (n=2). Staging included gastroscopy with multiple biopsies, endosonography of the upper gastrointestinal (GI) tract, computed tomography (CT) of thorax and abdomen, lymph node sonography, colonoscopy with multiple biopsies, otorhinolaryngologic assessment, MRI of salivary and lacrimal glands, and bone marrow biopsy. All lesions suggestive of lymphoma involvement were subjected to biopsy, if accessible, and biopsies were evaluated for MALT-lymphoma specific genetic aberrations by means of RT-PCR and/or FISH. These included assessment of t(11;18)(q21;q21), t(14;18)(q32;q21) involving IGH and MALT1, t(1;14)(p22;q32), t(3;14)(q14;q32) involving FOXP1 and IGH and trisomies 3 and 18. *Results.* Out of 140 patients, 52 (37%) were found to harbour multifocal MALT lymphoma involving multiple organs. In total, 15 of 61 patients with gastric lymphoma (25%) had multiorgan involvement. Eight out of these 15 patients showed synchronous spread to the GI tract (involvement of colon and/or rectum in 7 and small bowel in one patient), while 6 had disease at another non-GI site (for details see Table 1). Organs affected included the lung in three patients, and lung along with parotid, bladder plus spleen, kidney, lung plus bone marrow and bone marrow alone in one patient each. By contrast, significantly more patients with extragastric MALT lymphoma had dissemination to another MALT organ (37 of 79, 46%; p=0.045). Nine of these 37 patients had dissemination to the stomach. Out of these nine patients with secondary spread to the stomach, 4 had lymphoma originating in the lung, one each in the small intestine, the lacrimal gland, the parotid, the conjunctiva and the kidney, respectively. Only 3 of 140 (2%) patients had bone marrow involvement. T(11;18)(q21;q21) was significantly more common in gastric MALT lymphomas (p=0.002). The rate of both trisomies 3 and 18 was significantly higher in patients with extragastric lymphoma (p=0.003 for trisomy 3 and p=0.037 for trisomy 18), as was t(14;18) involving IGH/MALT1 (p=0.036). Multifocality was significantly associated with t(11;18)(q21;q21) in gastric lymphoma (p=0.045) and with trisomy 18 in extragastric lymphomas (p=0.011). *Conclusions.* Our findings suggest that MALT lymphoma frequently presents as a multifocal disease. Extragastric MALT-lymphomas are significantly more prone to dissemination than gastric MALT lymphomas.

1044

ANTIBIOTIC THERAPY WITH DOXYCYCLINE IS AN ACTIVE TREATMENT AGAINST OCULAR ADNEXA MALT LYMPHOMA: FINAL RESULTS OF A MULTICENTER PROSPECTIVE PHASE II TRIAL

J. M. Ferreri,[1] M. Ponzoni,[1] M. Guidoboni,[2] A. Giordano Resti[1], L. Politi,[1] S. Cortelazzo,[3] F. Zallio,[4] A. Palmas,[5] G. P. Dognini[1], E. Pasini,[2] F. Sacchetti,[2] C. De Conciliis,[6] C. Doglioni,[1] R. Dolcetti[2]

[1]*San Raffaele Scientific Institute, Milan, Italy;* [2]*Centro di Riferimento Oncologico, IRCCS, Aviano (PN), Italy;* [3]*Ospedale Riuniti di Bergamo, Bergamo, Italy;* [4]*Istituto Nazionale dei Tumori, Milan, Italy;* [5]*Ospedale San Francesco, Nuoro, Italy;* [6]*Ospedale San Giuseppe, Milan, Italy*

An association between ocular adnexal lymphoma of MALT-type (OAL) and Chlamydia psittaci (Cp) infection has been reported. Preliminary data suggest that patients (pts) with Cp-related OAL could achieve lymphoma regression after eradicating therapy with doxycycline, while data on the activity of this strategy in Cp-negative OAL are not available. In this multicentre prospective trial, 27 consecutive pts with OAL and measurable disease, at diagnosis (n=15) or relapse, were treated with doxycycline 100 mg, bid orally, for 3 weeks. Objective response was the primary endpoint. The presence of Cp DNA in lymphoma samples was evaluated by TETR-PCR. Tolerability was excellent in all pts but one. At a median follow-up of 13 months. (range 3-45), response was complete (CR) in 6 pts and partial in 7 (ORR=48%; 95%CI:30%-66%); three pts had a response <50%, 9 had stable disease (3-13 mo.), two had progressive disease. Response was slow; 5 pts achieved the best response only after one year of follow-up (median time to the best response: 6 mo.). TETR-PCR resulted positive in 11 (41%) pts and negative in 16. Lymphoma regression was observed in both PCR-positive and -negative pts (64% vs. 38%; p=0.25), with a CR rate of 36% and 13% (p=0.18), respectively. Response rate was similar between pts with conjunctival and intra-orbital lymphomas (43% vs. 54%, p=0.71). The three pts with regional lymphadenopathies and three of the 5 pts with bilateral OAL achieved objective response (4 CRs), which lasted 3+, 13+, 16+, 22+, 26+, and 37 mo. In relapsed pts, objective response was observed in 3 of 5 previously irradiated pts and in 5 of 7 non-irradiated pts (p=0.99). Twenty pts are failure-free, with a 2-yr FFS of 66±12%. Doxycycline is a fast, cheap and safe therapy, able to induce durable regression in 64% of Cp-related OAL. This antibiotic is a valid alternative against OAL, even in pts with multiple failures, involving previously irradiated areas or regional lymph nodes. We report for the first time responses also in PCR-negative OAL; this finding stimulates the development of more sensitive and specific methods for Cp detection and the study of potential associations with other infectious agents responsive to doxycycline.

Publication Only

1045

LONG-TERM USE OF OCTREOTIDE-LAR IN A PATIENT WITH TYPE 2B-VON WILLEBRANDS DISEASE, MULTIFOCAL ANGIODYSPLASIA AND MULTIPLE EPISODES OF GASTROINTESTINAL BLEEDING

C. Ulibarrena, J.L. Sastre, M.S. Garca-Torremocha

Complexo Hospitalario de Ourense, Ourense, Spain

The association between gastrointestinal angiodysplasia and von Willebrand's disease (vWD) is uncommon. Since the first description in 1967, some other 20 cases have been reported; most of them were vWD type 2 and 3. The efficacy of several therapies has been inconsistent and transient: transfusions, factor VIII / vWD concentrates, endoscopic sclerosis, estrogens, surgery. Bowers *et al*. published 2 cases with this association in whom a significant decrease in transfusion requirements and episodes of hemorrhage was obtained through the use of octreotide (*Br J Haematol. 2000 Mar;108(3):524-7*). The therapeutic effect of this synthetic analogue of somatostatin in this setting lies on the reduction of splanchnic blood flow to abnormal blood vessels. In one of their patients, an unforeseen increase in vW factor activity was also demonstrated. We describe an additional case with protracted use of octreotide in a long-acting formulation. 61 yr. old, female, allergic to iodine (anaphylaxia). vWD type 2b. Chronic hepatitis C of presumed post-transfusional origin, genotype 1. Multifocal angiodysplasia in gastrointestinal tract with recurrent episodes of upper and lower bleeding (>100 days of hospital stay and >100 blood products per year). Previous therapies: surgery (partial gastrectomy, hemicolectomy); combined estrogens and progesterone. She refused prophylactic use of vW / factor VIII derivatives.

Table 1. Evolution of parameter.

Years products	*Blood to hospital*	*Admittances of stay*	*Days*
1999	137	18	123
2000	168	22	167
2001	87	16	106
2002	131	16	122
2003	80	13	96
2004	77	9	90

In the second semester of 2000, we obtained permission for compassionate use of octreotide; the initial therapeutic scheme included a progressively higher dose to reach 250 μg SC t.i.d., even though compliance to side effects prevented a dose higher than 250 μg SC b.i.d. At the end of 2000, the conventional formulation was replaced by long-acting-release (LAR) octreotide, 20 mg IM monthly, each dose preceeded by 1000 IU of a factor VIII / vW concentrate to minimize local bleeding; both octreotide and factor were administered in the Day-In Hospital. The favourable evolution since then, with regard to decrease in number of hospital admittances, days of hospital stay and number of blood products transfused, is depicted on Table 1. In 2002, there was an apparent loss of response, related to a single episode of hemoperitoneum. In 2004, two of the admittances were due to thrombosis and infection of the central venous catheter, which required heparin therapy. When comparing data from 1999 and 2000 on one side, and those from the following 4 years on the other, the number of transfused blood products, hospital admittances as well as days of hospital stay, diminished by a gross one third (39%, 35% and 30%, respectively), with a trend to further decrease. In addition, this has led to a significant improvement in the quality of life for the patient, allowing her a 'life beyond the Hospital' The toxicity has been scarce, with a slight increase in blood pressure and glucose levels as the only relevant events. We believe that octreotide-LAR is an option to be considered in those patients with recurrent bleeding related to vWD and gastrointestinal angiodysplasia.

1046

PROPHYLACTIC TREATMENT WITH RECOMBINANT FACTOR VIIA (NOVOSEVEN) IN TWO PATIENTS WITH SEVERE CONGENITAL FACTOR VII DEFICIENCY

A. Gerhardt,[1] R.E. Scharf,[2] R.B. Zotz[2]

[1]*Universitätsklinikum Duesseldorf, Duesseldorf, Germany;* [2]*Universitätsklinikum Duesseldorf, Duesseldorf, Germany*

Backgrounds. Inherited factor VII (FVII) deficiency is a rare autosomal recessive disorder. Patients with severe homozygous FVII deficiency are at risk of umbilical and intracranial bleeding. In addition, FVII deficient patients can present with joint hemorrhage, muscle bleeding, menorrhagia, or mucocutaneous bleeding. Treatment of congenital FVII deficiency consists of replacement therapy with plasmic FVII concentrates or recombinant FVIIa (rFVIIa). Relatively small amounts of rFVIIa are required for replacement therapy in FVII deficient patients. Factor VII clotting activity levels of 15 to 25% of normal are considered to be sufficient for effective hemostasis in this patient group. Because of its short half-life of approximately 3 hrs., until now, rFVIIa has not been regarded as a routine prophylactic treatment option for FVII deficiency, although prophylaxis with rFVIIa has been described as an effective therapy in hemophilia A patients with inhibitory antibodies. *Case Reports*: We report on our experience of prophylactic treatment with rFVIIa in two FVII deficient patients. The first patient is a 43-year-old male with severe FVII deficiency (<1% activity) associated with compound heterozygous Gly78Asp and heterozygous Cys194Tyr mutations. After birth, he experienced severe tissue bleeding complications and later on various joint bleedings with consecutive hemophilic arthropathy. Initially he received on demand treatment with prothrombin complex concentrates and later regular prophylaxis with plasma derived factor VII. He is now on treatment with rFVIIa (1.2 mg 3 times per week) since 2 years. Since his substitution with rFVIIa, no spontaneous bleeding episodes occurred and no side effects were observed. The second patient is a 36-year-old male with FVII deficiency of <1% FVII activity. He experienced recurrent mucosal bleeding episodes and large skin hematomas. His genetic defect consists in a homozygous missense mutation (1061 C>T, Ala354Val), a heterozygous missense mutation (218 T>A, Leu73Gln), and a heterozygous frameshift mutation (1391delC, Pro464HisfsX32). The patient is on prophylactic treatment with rFVII (1.2 mg 3 times per week) since 18 months. Again, since substitution with rFVIIa, no spontaneous bleeding episodes occurred and no side effects were observed. In conclusion, prophylactic treatment with rFVIIa three times per week in FVII deficient patients appears to be an effective and safe therapeutic option. The mechanisms by which rFVIIa prevents bleeding episodes despite a short half-life of 3 hrs. remains to be elucidated.

1047

EFFICACY OF RITUXIMAB TREATMENT IN POST-PARTUM ACQUIRED HEMOPHILIA

C.S. Santoro, S. Bernasconi, M.S. De Propris, A. De Vellis, E. Murgi, A. Rago, F. Torelli, R. Foà, M.G. Mazzucconi

Hematology, ROMA, Italy

Background. Selective B cell depletion following Rituximab treatment, has been shown to be an effective strategy for the therapy of immune disorders. *Aims*. To evaluate the efficacy of Rituximab in patients with high titer post-partum acquired inhibitor against factor VIII resistant to several therapy lines. Patients' history and results. A 25 years old woman, with a previous diagnosis of post-partum acquired hemophilia A, came to our Institution in December 2002 because she was not responsive to prednisone (1 mg/kg/day). The aPTT ratio was 2.59; FVIII:C <1%; inhibitor titer 621 BU/mL. The patient was subsequently treated with dexamethasone (40 mg/day for 4 days), immunoglobulins (2 g/kg/ for 4 cycles), oral cyclophosphamide (100 mg/day for two months) without any response. Intercurrent hemorrhagic events were treated with rFVIIa. In July 2004, a sudden abdominal blood shedding was diagnosed (hemorrhagic corpus luteum) and the patient was treated with rFVIIa (90 μg/kg every 3 hours) and red blood cell transfusions. In August 2004, she began therapy with Rituximab (375 mg/m^2/once a week for four doses). Inhibitor titers were as follows: pre-Rituximab, 206 BU/mL; first week after therapy 75 BU/mL; first month 50 BU/mL; third month 9.7 BU/mL; fifth month 2.1 BU/mL; seventh month 0.71 BU/mL; ninth month 0 BU/mL; twelfth month 0 BU/mL. FVIII:C values: third month 1.2%; fifth month 11%; seventh month 20%; ninth month 40%; twelfth month 46%. CD19+ B cell values: pre-Rituximab, 205×10^6/L; first week after therapy, 0; fifth month, 0; ninth month, 56×10^6/L; twelfth month, 212×10^6/L. Since the start of Rituximab, the patient experienced no hem-

orrhagic events. *Conclusions*. Rituximab therapy is effective in the treatment of acquired hemophilia A with high titer inhibitor resistant to other immunesuppressive therapy lines.

1048

DYSKERATOSIS CONGENITA: UNUSUAL PRESENTATION WITH TROMBOCYTOPENIA IN EARLY AGE

K.S. Kocheva,[1] M.O. Muratovska,[1] G.S. Glamocanin[1], M.K. Martinova,[1] A.Z. Antevska T.,[1] P. Tsaftaridis[2]

[1]*Pediatric clinic, Skopje, Macedonia;* [2]*National and Kapodistrian University of Athens, Greece*

Backgrounds. Dyskeratosis congenita (DC) is rare, usually fatal inherited skin and bone marrow (BM) failure syndrome that displays considerable genetic and clinical heterogeneity. Classical DC is an inherited BM failure syndrome characterized by the mucocutaneus tried of abnormal skin pigmentation, nail dystrophy and mucosal leucoplakia A variety of the other somatic abnormalities have also been reported. At genetic level X-linked recessive, autosomal dominant (AD) and autosomal recessive (AR) forms of the disease exist where the genetic basis of the X-linked and AD forms have been determined. The X-linked form of DC is due to mutations in DKC1, the gene encodes dyscerin, a protein that is part of telomerase complex. Autosomal dominant DC is caused by mutations in TERC, which codes for the RNA component of telomerase. The DKC1 gene is expressed in all tissues of the body consistent with it having a *house keeping function* in the human cell. This correlates well with the multi system phenotype of DC. *Aims.* In this report we present unusual onset of DC with trombocytopenia as a first presentation at an early age. *Methods/Results.* A 4 years old male patient with DC, presented at the age of 18 months with isolated trombocytopenia presiding characteristic skin finding and nail dystrophy. The trombocytes count of 24. 000/mm^3 persisted without the presence of anemia or Neutrogena Trombocytopenia responded to kortikosteroides at a dose of 2.5 mg/kg per day. Six mounts late he was admitted in the hospital with sever anemia; trombocytopenia and neutrogena Severe aplastic anemia was later diagnosed. Clinical examination showed hyperpigmentation over the neck, and nails dystrophy. All the nails were dystrophic. To substantial the diagnosis the genes responsible for the X-linked and AD forms of DC (DCK1 and TERC) were screened for mutations. No abnormal patterns have been detected in patient in either gene. However, his mother appears to have a highly skewed pattern of X- chromosome inactivation that is characteristically observed in carriers of DKC1 mutations. *Conclusions.* This patient's clinical course is interesting because the thrombocytopenia developed as an isolated symptom at the age of 18 months and preceded the skin anomalies. The diagnosis of dyskeratosis congenita was made only after an evolution. The diagnosis of dyskeratosis congenita, should be considered in every child first seen with thrombocytopenia or aplastic anemia. In some patients the BM abnormalities may appear before the mucocutaneous manifestations and can lead to an initial diagnosis of 'idiopatic aplastic anemia'

1049

SUCCESFULL TREATMENT OF RECOMBINANT FACTOR VIIA OF THERAPY RESISTANT LIFETHREATENING BLEEDING IN TWO PATIENTS WITH THROMBOCYTOPENIA

M. Akbalik,[1] T. Fisgin,[2] F. Duru,[2] M. Tosun,[3] D. Albayrak[2]

[1]*Ondokuz Mayis University, Samsun, Turkey;* [2]*Omu Faculty of Med.Dep.Ped. Hematology, Samsun, Turkey;* [3]*Omu Faculty of Med.Dep.Obstet.Gyn., Samsun, Turkey*

Thrombin formation has a crucial role in providing homeostasis. The hemostatic effect of activated recombinant FVIIa (rFVIIa) is mediated by an enhanced rate of thrombin generation. Recombinant FVIIa was developed initially for treatment of bleeding in Hemophilia A patients with inhibitors. Thereafter it was recognized that rFVIIa (NovoSeven) was a very valuable haemostatic agent also for different bleeding disorders such as nonhemophilic patients with acquired antibodies against rFVIII, congenital FVII deficiency, uncontrolled bleeding due to thrombocytopenia and functional platelet defects. Here we report the successful use of an activated recombinant factor VIIa in two patients with severe bleeding caused by therapy resistant thrombocytopenia. Patient 1; A 14 year old girl with Evans syndrome was unresponsive to the repeated therapy of immunoglobulin and corticosteroids for six years. She was recently presented with somnolence, severe headache, nausea and vomiting. The cranial computerized scan revealed left frontotemporal hemorrhage. Her initial platelet count of 2500/mm^3 was increased up to maximal 10-15,000/mm^3 despite vigorous platelet transfusions. Since we failed to achieve homeostasis we used rFVIIa at a dose of 100 microg/kg along with platelet suspensions which was followed by 50 microg/kg repeated doses at 2-h intervals for two times. The bleeding was taken to control and the symptoms of the patient improved gradually and she was discharged without any sequel. Patient 2 was an 18 year old girl with therapy resistant Fanconi's Anemia in whom platelet refractoriness was developed. She suffered from severe life-threatening menorrhagia. In this patient menorrhagia could not be controlled by repeated thrombocyte transfusions and oral contraceptive therapy. Recombinant FVIIa at initial dose of 90 microg/kg was given and it was repeated at the same doses at 2-h intervals for two times. Her hemorrhage decreased gradually and ceased completely after the third injection of rFVIIa. No side effects related to use of rFVIIa has been observed in these two patients. We concluded that recombinant factor VIIa should be considered as a therapy choice in life-threatening bleeding of the patients with therapy resistant immune thrombocytopenia and aplastic anemia in whom thrombocyte refractoriness is developed.

1050

BLOOD IN URBAN MULTIPLE CASUALTY INCIDENTS: THE EXPERIENCE OF A LEVEL 1 TRAUMA CENTER IN ISRAEL

O. Ben-Tal,[1] J. Klausner,[1] D. Bar-Zohar,[1] O. Szold,[1] C.I. Schulman,[2] P. Halpern,[1] A. Shimonov,[1] M. Hareuveni,[1] D. Soffer[1]

[1]*Tel-Aviv, Sourasky, Medical Center, TEL-AVIV, Israel;* [2]*Trauma and Critical Care, Miami, Florida, USA*

Background and Objectives: The issue of blood utilization and blood bank operating protocol in the setting of multiple casualty incidents (MCI) has not been elucidated. The objectives of the current study were to analyze the pattern of blood ordering and administration as weel as the timing and type of components, during MCIs, in order to determine the framework needed for the blood bank in a level I Trauma Center in the setting of such events. *Methods.* A retrospective study evaluating data collected in 18 consecutive terrorist attacks in the city of Tel-Aviv between January 1997 and February 2005. Data were retrieved from chart review and from the blood bank and emergency department (ED) computerized MCI registry programs and analysed by size of MCI, type and severity of victim injury, timing and type of blood and components. *Results.* Three hundred thirty two packed red blood cell (PRBC) units were transfused altogether, half of which were administered in the setting of massive transfusion (>10 PRBC units per 24 hours) to 4.7% of the patients. The ratios of transfused PRBC units per evacuated and admitted patients 'packed cell per patient index (PPI) - were 0.58±0.98 and 1.1±1.5, respectively. The PPI rose significantly when there were over 25 evacuated victims (p=0.039). The most frequent major blood group transfused was O (Rh positive and negative), 57% of all transfused PRBCs, about half of which were administered urgently, untested. Half of the blood units were supplied during the first two hours post admission. TASMC blood bank standard operating protocol (SOP) includes sending a blood bank liaison to the ED and OR to coordinate activity and supply urgent, untested, group O PRBC units. *Conclusions.* Roughly, one unit of blood per admitted victim in a small MCI and 2 units in a large MCI, half of which are group-O, may serve as a basic estimate of the transfusion needs in the first hours after urban MCIs. Blood bank operations must be coordinated with the other medical teams dealing with a MCI.

1051

INVESTIGATION OF PRIMARY HEMOSTASIS USING PFA-100 ANALYZER

M.N. Blazek, M. Blaha, J. Maly, V. Blaha, M. Cermanova, M. Pecka, L. Slovacek

Faculty Hospital, Charles University, HRADEC KRALOVE, Czech Republic

Backgrounds. LDL-apheresis is a method of extracorporeal elimination of serum LDL-cholesterol that is used for treatment of patients with severe hyperlipidemia resistant to diet and pharmacotherapy. Applicable markers that could be used to determine efficacy of this treatment to lower the activity of atherosclerosis are still to be found and remain unresolved. Activity of primary haemostasis plays an important role in the development of atherosclerotic complications. *Aims.* We hypothesize that investigation of primary haemostatic activity could be a quick and useful marker for monitoring LDL-apheresis efficacy. The aim of this work was to verify this hypothesis. *Methods.* Commercial analyser Dade Behring PFA-100, Germany (PFA, platelet function analyse) was used

for all investigations. This analyser enables quantitative measurement of platelet-mediated haemostasis in noncoagulable (citrated) blood. The method simulates platelet activation by mechanical stress - shear stress, and also simulates contact of platelets with collagen. There were 9 patients with familiar hypercholesterolemia in the study group (4 females and 5 males). Age ranges from 17 to 59 years (46,4 years average and 55 years median), 2 of them have homozygous hypercholesterolemia. Our aim was to investigate the changes before and after procedure two times in every patient. *Results.* 18 pairs of samples were examined using COL/EPI membrane (collagen/epinephrine) and 17 pairs of samples were examined using COL/ADP membrane (collagen/ADP), total number of samples was 70. Closure time (CT) values were prolonged after separation in all cases but CT prolongation was not statistically significant (p<0,14). No differences between homozygous and heterozygous patients were found. *Summary/Conclusions.* Investigation of primary haemostasis immediately after procedures using PFA-100 analyser is not a suitable marker and could not be used to determine the optimal intensity of particular LDL-apheresis procedures.

Funding: MZ CR MZO 00179906.

1052

TWINING IS A SIGNIFICANT INDEPENDENT FACTOR IN THE DEVELOPMENT OF CHILDHOOD MALIGNANCIES

M. Moser,[1] Y.B. Bodenheimer,[1] A.C. Cohen,[2] I.H.V. Har-Vardi,[3] J.K. Kapelushnik[1]

[1]*Soroka Medical Center, Beer Sheva, Israel;* [2]*Clalit Health Services, Beer Sheva, Israel;* [3]*Reproduction Unit, Soroka Medical Center, Beer Sheva, Israel*

In the past few years we have noticed a marked increase in the incidence of cancer among twins treated at the Pediatric Hematology-Oncology Unit (PHOU) of Soroka Medical Center (SMC) in Beer-Sheva, Israel. In this work, we reviewed the relationship between twinning and the risk of developing cancer during early childhood. Children born at SMC between Jan. 1991 and Sep. 2003, with any malignancy were included in the study if they were under 13-years-of-age at time of diagnosis. Three controls of the same sex were matched to each patient from the birth registries of the same day at SMC. A twin was not chosen as a control to its sibling. Data from patients, controls, and mothers were collected from medical records and included three areas of investigation: demographics and obstetric history of the mothers, delivery data, and gestational events and/or interventions (infertility including *in vitro* fertilization (IVF), ART, diabetes mellitus (DM), hypertension (HTN), urinary tract infections (UTI), iron deficiency anemia (IDA), and medications). A total of 143,087 deliveries, resulting in 145,503 children, were registered at the Soroka Medical Center (SMC) between January 1991and September 2003. Of those, 98.37% were singleton and 1.63% were multiple births (2,261 twins; 77 triplet and quadruplets), cumulating in 972,000 patient years of follow up. The crude incidence of cancer during childhood is 14:100,000 per year, while the incidence of cancer calculated for the children born during the study period was 10.5:100,000 per year. Of the 92 children with cancer, complete information was obtained for 65 patients (70.6%); eight (12.3%) were twins, four were born after ART (6.7%), two of whom were twins. Significantly more Bedouin children were found in the patients group. According to this data, the total expected number of cancer cases among twins born during the study period was 2.23, while the total observed number at the PHOU is 3.58 times higher (8 cancer cases), p<0.001. Twining *per se* was found to be an independent factor in the development of childhood cancer. Although seeming significant in a univariate analysis, this study cannot point to a significant multivariate correlation between ART and childhood cancer (p=0.07), most likely due to the small sample size. More children of Bedouin origin were found in the patients group possibly due to higher consanguinity rate and/or low socioeconomic status. However, to date, no studies have addressed this matter and more investigation is needed.

1053

SERUM AND SALIVARY IRON AND FERRITIN LEVELS IN PATIENTS WITH THALASSEMIA

D. Canatan, S. Kosaci Akdeniz

Sleyman Demirel University, Isparta, Turkey

Backgrounds. The thalassemias are a group of autosomal recessive blood diseases of varying degrees of underlying genetic defects that include total or partial deletion of globin chains and nucleotide substitutions, deletions or insertions. Current therapy includes regular blood transfusions and iron chelation. Chronically increased iron load is due to excessive hemolysis, increased intestinal iron absorption and frequently blood transfusions that causes organ damage and dysfunction. Especially in childhood period, serum iron level measurement methods are tecnically invasive. Difficulty of the current methods used to evaluate the iron accumulation in organs suggests the importance of saliva usage for diagnosis. In this study, it has been supposed that salivary iron amount could be a marker of total body iron storage in patients with thalassemia. *Material and Methods.* 34 healthy children as control group were compared with 71 thalassemia major, 10 thalassemia intermedia and 15 thalassemia trait. Salivary and serum iron and ferritin levels were measured in all groups. *Results.* there was no statistically significant difference between the control group and other gorups by means of age and gender (p > 0.05). There was a correlation between serum and salivary iron and ferritin levels in thalassemia major, intermedia and trait groups (Table)

Table. Correlation between serum and salivary iron and ferritin levels in patients thalassemia.

	Salivary and Serum Iron	*Salivary and Serum Ferritin*
Control	r =0.885, p=0.000*	r =0.842, p=0.000*
T. Major	r =0.972, p=0.000*	r =0.364, p=0.034*
T. Intermedia	r =0.720, p=0.019**	r =0.891, p=0.001**
T. Trait	r =0.955, p=0.000**	r =0.831, p=0.000**

As a conclusion, salivary iron and ferritin levels increases as well as serum levels. This increasment in salivary iron amount may be an indicator of total iron accumulation. Therefore non invasive, salive samples for measurement of iron and ferritin may prefer instead of blood samples in patients with thalassemia. For this reason, we think that more extensive and controlled studies are needed to use the saliva as a routine diagnostic material.

1054

FERTILITY AND REPRODUCTION IN THALASSEMIA MAJOR

A. Meo,[1] L. Zanghì,[2] M.A. La Rosa,[2] R. Silvestri[3]

[1]*Policlinico 'G. Martino' Medical School, Messina, Italy;* [2]*Department of Paediatric Sciences, Messina, Italy;* [3]*Department of Neurosciences, Psychiatric, Messina, Italy*

Backgrounds. Therapeutic advances in thalassemia major have increased the average lifespan and improved the quality of life patients. Attainment of reproductive capacity and creation of a family has become a challenging task. Hypogonadotrophic hypogonadism due to hemosiderosis is still present and become a barrier in their desire for parenthood. Nowaday women with thalassemia can safely complete pregnancy, but the decision to conceive has to be carefully considered by a couple in consultation with their doctors. Patients with thalassemia who have a normal menstrual cycle may conceive spontaneously. Those suffering from primary or secondary amenorrhoea can be submitted with hormonal treatment in order to stimulate the production of ova and the induction of ovulation. *Aims.* Aim of our study is to exstimate the frequence of fertility (spontaneous or after induced ovulation) and pregnancy complications for mother and newborn, in patients admitted to the Paediatric Department- Thalassaemia Ward *G. Martino* Policlinico. *Patients and Methods.* We followed 36 women mean age 32 (18-46) years. All patients were treated according to the standard treatment protocol. 9/36 women with Thalassemia Major became pregnant and were the object of our study. At the beginning of pregnancy, average age was 26 years. Five pregnancies were spontaneous and four were induced after ovarian stimulation followed by natural insemination. Women who expressed the desire to become pregnant underwent a complete evaluation of psychological and clinical conditions. Glucose tolerance, tiroid, serum ferritin levels, hepatic and renal function tests, bidimensional echocardiography were performed before, during the pregnancy and after delivery. Also Bone Mineral Density (BMD) was measured, by the DEXA method, before pregnancy and after delivery. Once the patients were confirmed to be pregnant, iron chelator treatment was stopped. Mean pre-transfusional Hb and blood consumption have been monitored in order to keep pre-transfusional Hb at 10-10,5 g/dl levels. A complete obstetrical survey was performed every two weeks. *Results.* Our findings show that 8 babies were delivered by elective caesarean section at 37° weeks of gestational age (GA). The mean birthweight of the newborns was 2954 g. All babies were normal. Ferritin levels increased during pregnancy in all

patients. After delivery all of them were in good general conditions and were treated with intensive iron chelation in order to reduce iron overload. No changes in laboratory parameters, BMD, ecocardiography evaluation hepatic and renal functions, have been observed besides increased iron stores. There were no delivery complications but one case of intrauterine death at the 35° weeks due to acute placental injury and one abortion in the early pregnancy were reported. *Conclusions.* Pregnancy can be safe in mothers and babies if closely monitored. Reproduction in patients with thalassaemia major is becoming a new reality, allowing them an improved quality of life. Maternity desire has to be considered with special caution and sensitivity. An optimal relationship has to be reached beetwen patients and care-givers to improve patients' safety. This demands a complex effort and embraces all disciplines and sectors requiring a comprehensive, multifaceted approach to identify and manage actual and potential risks.

1055

EFFICACY AND SAFETY OF INTRAVENOUSLY ADMINISTERED IRON SUCROSE (VENOFER) IN IRON DEFICIENCY ANEMIA PATIENTS

A. Salamat, L. Wakeman, R. Munro, L. Davey, E. Lardner, J. Daly

Swansea NHS Trust, Swansea, United Kingdom

Conventional oral iron administration in iron deficiency anaemia (IDA) patients is simple, effective and tolerable. Nevertheless, there are patient cohorts in which an alternative method of iron delivery is required. These groups include patients with inflammatory bowel disease related malabsorption and bleeding, end stage renal patients receiving erythropoietin, patients with unresolved ongoing bleeding requiring in excess of the acceptable oral dose and patients exhibiting severe gastrointestinal adverse effects to oral iron. Intravenous iron administration provides a simple, practical alternative for these patients allowing the deliverance of far greater doses than the oral route. Reluctance in prescribing this treatment results from previously reported serious adverse effects of iron dextran. We present retrospective data from 53 episodes of IDA patients (N=47) treated with intravenous iron sucrose (Venofer). The objective of this retrospective study was to determine the efficacy and safety of Venofer in IDA patients unable to tolerate oral iron administration. A total of 47 IDA patients (Number of episodes (NE)=53; F=41; M=12) whom were clinically assessed to require an alternative route of iron administration received appropriate doses of Venofer (1=2000 mgs; 1=1600 39=1000 mgs; 3=800mgs; 3=400 mgs, mean dose=964 mgs). Mean ages were 57 and 60 years for males and females respectively.

Table 1. Mean differences and T-test *p*-values.

Condition	*Mean improvement*			
	Hb (g/dL)	*FERR (µg/dL)*	*MCV (FL)*	*MCH (pg)*
ALL patients	2.0 (p<0.001)	113.5 (p<0.001)	6.9 (p<0.001)	2.8 (p<0.001)
ACD	1.12 (p=0.047)	128.8 (p=0.011)		
CKD	2.58 (p=0.008)	154.8 (p=0.008)		
GAST	2.01 (p<0.001)	69.5 (p<0.001)		
Menor	3.0 (p<0.012)	118.3 (p<0.022)		

Patients were anaemic due to either anemia of chronic disease (ACD) (F=9; M=0), chronic kidney disease (CKD) (F=3; M=3), gastrointestinal related diagnosis (GAST) (F=22; M=9) or menorrhagia (MENOR) (N=7). Haemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH) and ferritin (FERR) parameters were determined pre- and post-Venofer infusion and results analysed statistically to determine mean improvement, normality and significance. Laboratory results for the whole data set (table 1) were analysed statistically using T-tests of mean difference. Results show an overall statistically significant improvement in Hb (2.0g/dL), FERR (113.5µg/L), MCV (6.9FL) and MCH (2.8pg). Results categorised according to IDA precipitating condition show significant differences between Hb and FERR measured pre- and post-Venofer administration in all cohorts. The greatest improvement in Hb and FERR were seen in the MENOR and CKD patient groups respectively. Venofer was well tolerated, one patient was excluded after the first dose due to onset of nausea, vomiting, tachycardia and a slight drop in systolic blood pressure. Intravenous Venofer administration is a well tolerated, effective alternative to oral iron in IDA patients, particularly in IDA secondary to menorrhagia and CKD. The mean Hb difference rises by 0.003g/dL and mean FERR by 0.09 µg/L for every 1mg increase in Venofer dose. Implications for the patient and the clinician include training on self administration for safe, effective home therapy.

1056

POST-TRAUMATIC STRESS DISORDER IN CHILDREN AFFECTED BY SICKLE CELL DISEASE AND THEIR PARENTS

M. Hofmann, M. de Montalebert, B. Beauquier-Maccotta, B. Golse

Hopital Necker, Paris, France

Backgrounds. Children affected by SCD suffer from recurrent painful crises, some of them being life threatening, or felt as life threatening by children and/or their parents. *Aim.* We hypothesized that painful crises could generate PTSD in some children, PTSD being described in patients having experienced or witnessed an event that involved an actual or threatened injury to physical integrity of self or others. Patients with PTSD present symptoms in each of these categories: reexperiencing, avoidance/numbing, increased arousal. Until now, PTSD had never been described in SCD children. *Methods.* We enrolled 11 SCD children, 9 males, 2 females hospitalized at least once for a painful crisis, at least one month before the study, who accepted with one of their parents to participate in the study. Their mean age was 11.8±2.2 years (range: 7.5-15.5). One father and 10 mothers were also studied, one mother being secondarily excluded from analysis because she did not answer the questions. Both children and parents had to answer to a semi-structured interview (SCID) and to complete questionnaires (IES-R, STAY-Y, BDI-II, CDI, CBCL). Socio-demographic data and medical past histories were registered. Statistical analysis used exact Fischer test and Mann Whitney test. Results. Three children had a PTSD (27%), and 4 parents (40%). We found no correlation between PTSD and socio-demographic data. Mean numbers of hospitalization/year were respectively 1.12±0.5 and 1.30±0.5, and mean number hospitalization in intensive care units respectively 0.33±0.6 and 0.5±0.8 in children with and without PTSD (N.S.). PTSD presence was not correlated in children and parents. There was a correlation between PTSD and the parents' feeling of powerlessness on their child's illness (p: 0.04). *Summary/Conclusions.* Painful crises are the most frequent complications of SCD, and one of the most difficult question for their management is the assessment of pain intensity. We show here that PTSD could be a complication of SCD. Symptoms such as intrusive distressing recollections of past painful events may worsen the consequences of vasoccclusion, enhancing children's feeling of pain, and leading physicians to an inadequate use of analgesics instead of psychological support. Furthermore, stress is a precipitating factor for vasoocclusive crises and may facilitate recurrences of painful episodes. Moreover, PTSD itself causes psychological distress and may disturb children's development. Interestingly, PTSD was not related in our study to the objective severity of the disease. Looking for PTSD and proposing specific individual and family psychological interventions could very probably contribute to disrupt the vicious circle between pain and fear of pain in SCD children.

1057

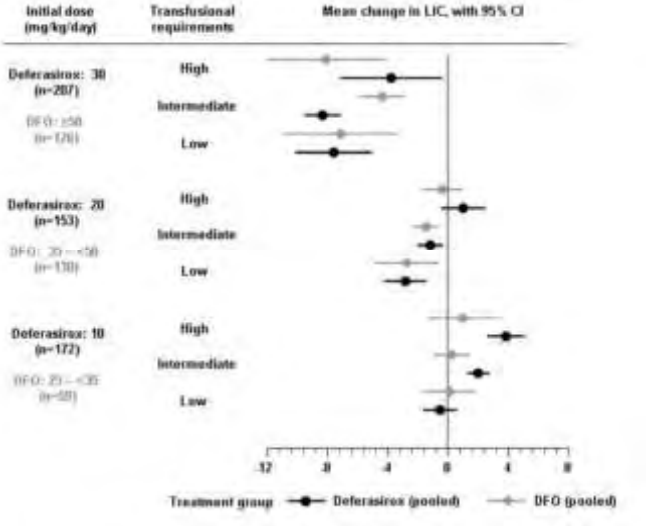
TRANSFUSIONAL REQUIREMENTS ARE A KEY FACTOR IN TAILORING THE OPTIMAL DOSE OF CHELATION THERAPY, AS DEMONSTRATED BY THE NOVEL, ORAL IRON CHELATOR DEFERASIROX (EXJADE, ICL670)

A. Piga,[1] Y. Aydinok,[2] I. Gathmann,[3] H. Opitz[3]

[1]*University of Turin, Turin, Italy;* [2]*Ege University Medical Faculty, Izmir, Turkey;* [3]*Novartis Pharma AG, Basel, Switzerland*

Backgrounds. The novel, once-daily oral iron chelator deferasirox (Exjade®, ICL670) has recently been approved in eight countries, including the USA and Switzerland, for the treatment of chronic transfusional iron overload in patients aged ≥2 years. Data from deferasirox clinical trials demonstrated a clear dose response, allowing physicians to tailor the dose to meet a patient's therapeutic goal, ie maintenance or reduction of iron burden. A high transfusion rate indicates that excess iron is more likely to accumulate, therefore further analyses were performed to evaluate the impact of ongoing transfusions as a determining factor in the management of patients receiving chelation therapy. Aims. The aim of this post-hoc, cross-study analysis was to evaluate the change in body iron burden stratified according to the transfusional requirements of iron overloaded patients during treatment with deferasirox or deferoxamine (DFO, Desferal™). *Methods.* Body iron burden was evaluated by pooling liver iron concentration (LIC) and serum ferritin data from four pivotal deferasirox clinical trials of up to 1 year's duration. In total, 1,005 patients with a variety of transfusion-dependent anaemias were enrolled; 652 were randomized and treated with deferasirox, 353 were randomized and treated with DFO. All patients were stratified into three categories depending on their transfusional requirements while on study: <7 (low), 7-14 (intermediate) or >14 (high) mL/kg/month of

packed red blood cells; 7 and 14 mL/kg/month correspond with approximately 2 and 4 adult units of blood, respectively. *Results.* In the overall population, 146 (22.4%), 419 (64.3%) and 87 (13.3%) patients who received deferasirox while on study had low, intermediate and high transfusional requirements, respectively; the equivalent numbers for patients receiving DFO were 61 (17.3%), 235 (66.6%) and 57 (16.1%), respectively. In completing patients with an LIC assessment at baseline and study end (approximately 90% of the overall population), a transfusion- and dose-related pattern was observed in the response of LIC (Figure 1) and serum ferritin. This was observed for both deferasirox (n=566) and DFO (n=327). Changes in iron burden were similar at comparable therapeutic doses. *Conclusions.* Transfusional requirement has a clear impact on response to chelation therapy. Physicians are able to tailor deferasirox dose according to patient needs, with dosing based on transfusion rate, severity of iron overload and treatment goal. Using this method, deferasirox was shown to meet the individual requirements of an extremely high proportion of patients treated. Deferasirox 10 mg/kg/day maintained iron balance in patients with low transfusional requirements, 20 mg/kg/day maintained or reduced iron balance in patients with low and intermediate requirements, while 30 mg/kg/day reduced iron balance in patients with high transfusional requirements. As regular transfusions lead to rapid iron accumulation, it is essential to monitor patients for the number of blood units transfused, serum ferritin levels and/or LIC. Across a range of transfusion-dependent anaemias and transfusional requirements, DFO and deferasirox in a 2:1 dose ratio have comparable effects on LIC and serum ferritin.



**Data not shown for the deferasirox 5 and DFO <25 mg/kg/day dose cohorts due to low patient numbers in these cohorts.*

Figure 1. Change in LIC (mg Fe/g dw), by treatment, dose and transfusional requirements.*

1058

IRON OVERLOAD CAUSED BY REPEATED TRANSFUSIONS IN ADULT CHRONIC REFRACTORY ANEMIAS

B.S. Kim,[1] I.-H. Kim,[1] S.S. Yoon,[1] J.S. Lee,[1] Y.Y. Lee,[2] S.Y. Park[1], B.K. Kim,[1] K.S. Han[1]

[1]*Seoul National University Hospital, Seoul, South-Korea;* [2]*Han Yang University Hospital, Seoul, South-Korea*

Many patients with refractory anemia suffer from iron overload and associated complications. However, it is not yet established when to start deferoxamine therapy to prevent this. To analyze the clinical features of iron overload caused by repeated transfusions in adult chronic refractory anemias. We chose patients who received more than ten units of RBC cells from the database of blood bank in our institute and their medical records were retrospectively reviewed. Twenty-nine patients (M: 18, F: 11) were identified and median age was 52 years (range: 22-82). Underlying disease causing repeated transfusions was aplastic anemia in 11 patients, myelodysplastic syndrome in 9, idiopathic myelofibrosis in 2 and multiple myeloma in 2 patients. Each of the remaining patients had pure red cell aplasia, chronic lymphocyte leukemia, non-Hodgkin's lymphoma, chronic myelogenous leukemia and acute myelogenous leukemia. Patients received median 85 units of RBC transfusions (range: 26-226). Two patients received 45, 101 units of RBC transfusions and developed liver cirrhosis after 143, 184 months from the initial diagnosis of their underling diseases, respectively. Cardiomyopathy developed in 4 patients after 29, 100, 104, and 143 months from the diagnosis of underlying diseases. They received 71, 103, 115 and 133 units of RBC, respectively. Diabetes mellitus developed in 5 patients. Twenty-four patients started deferoxamine therapy when they received median 48 units of RBC (range: 18-164). Eight patients already had complications (liver cirrhosis: 2, cardiomyopathy: 2, diabetes mellitus: 4) at the time of starting deferoxamine. Eleven patients received treatment for underlying disease on a curative intent (allogeneic stem cell transplantation, 7; autologous transplantation, 1; combination chemotherapy, 3), but only three of them responded. Four patients died of underlying diseases and three patients died of complications associated with treatment for underlying disease. Two patients died of cardiomyopathy. Median overall survival after the diagnosis of underlying disease was 150 months. Iron overload is a common complication of adult chronic refractory anemia. The risk of developing serious complications increased with the increase of RBC transfusions. Patients who developed cardiomyopathy had a worse prognosis especially and therapy should be started earlier to prevent associated complications.

1059

FERRITIN LEVELS, NON-COMPLIANCE AND ADVERSE EVENTS IN RELATION TO INFUSED IRON CHELATION THERAPY IN AN INTERNATIONAL COHORT OF PATIENTS FROM ACTUAL PRACTICE

K. Payne,[1] M.P. Desrosiers,[1] I. Proskorovsky,[1] K. Ishak,[1] J.F. Baladi[2]

[1]*Caro Research Institute, Montreal, Canada;* [2]*Novartis Pharmaceuticals Corporation, Florham Park, USA*

Background. Deferoxamine (DFO) is an iron chelation therapy (ICT) administered to patients undergoing chronic blood transfusions. Although efficacious, it is burdensome to patients because of the necessity of almost daily infusions lasting 8 to 10 hours each and the occurrence of treatment related adverse events. Non-adherence to ICT, however, results in iron overload which, if not removed, results in serious clinical and economic outcomes such as myocardial, endocrine and hepatic dysfunction. *Aims*. To document ferritin levels, non-compliance and prevalence of adverse events in a cohort of patients undergoing infused ICT. *Methods*. A retrospective, semi-prospective study of the economic and quality of life burden of infused ICT in the usual care setting was undertaken. Compliance and adverse events were obtained from patient interviews. Serum ferritin level data and adverse events experienced by these same patients during their initial and most recent year of ICT therapy were collected from the patients' medical charts. *Results*. 78 patients (44% male; mean age: 28.9 ± 14.6 years) with thalassemia (n=51), sickle cell disease (n=23), and myelodysplastic syndrome (n=4) were recruited from 8 different sites within the US (4 sites) and the UK (4 sites). Sixty per cent of patients reported non-compliance to ICT over the previous week. Of these, 43% could be considered at risk for iron overload complications because they reported missing more than 2 out of 5 doses. Over the previous 30 days, 64% of patients suffered at least one adverse event; those most commonly reported were site soreness (86%), site irritation, (74%), ringing in the ears (22%) and abdominal pain (20%). Of the 56 (75%) patients who had missed at least one dose during the past 4 weeks, 23% did so because of adverse events to ICT. During the initial year of ICT, the adverse events documented in the charts of 8 patients were injection site soreness/rash (50%), allergic reaction to medication (13%), breathing problems (13%) and nausea (13%) while in the most recent year of ICT, the adverse events most commonly reported by 13 patients were injection site soreness/rash (31%), tinnitus (13%) and joint pain (13%). Serum ferritin level test results obtained from charts indicate that, in general, average blood iron levels are somewhat high and increase slightly over time despite ICT. In some patient categories, this increase is more pronounced. For the initial year of ICT, the mean serum ferritin level was 2,618±1,377 ng/mL (US:2,519±1,382; UK:3,013±1,370) and 2,766±2,272 ng/mL (US:2,741±2,532; UK:2,813± 1,740) for the most recent year. For patients for whom data of the most recent year and the first year of ICT were available, the mean serum ferritin level increased by 394.1± 2,633 ng/ml (US: 306±2,774; UK:1,029±1,225) over that time period. *Summary/conclusions*. Current chelation therapy may not provide adequate effectiveness in the real world. Non-compliance to infused ICT is prevalent indicating the prognosis for the patient will decline. The occurrence of bothersome side effects may contribute to this observation. A novel ICT agent with improved tolerability could improve the clinical and economic outcomes of therapy.

1060

RESPONSE RATE IN IDIOPATHIC THROMBOCYTOPENIC PURPURA. SINGLE INSTITUTION EXPERIENCE

I. Macarie,[1] G. Oltean,[1] S. Demian,[1] M. Macarie,[2] M. Candea,[1] B. Dorcioman[2]

[1]*Clinica Medicala [1], TARGU MURES, Romania;* [2]*Spitalul Clinic Judetean de Urgenta, TARGU MURES, Romania*

Backgrounds. Idiopathic thrombocytopenic purpura (ITP), also reffered as immune or autoimmune thrombocytopenic purpura, is an acquired disease characterized by low platelet count, normal bone marrow, usually with an increased number of megakaryocytes and the absence of any other disease. The majority of patients respond, on short term, at an initial corticosteroid therapy, but this approach does not influences the natural evolution of the disease on long term, as only about a third of the patients remain in sustained remission at the cessation of treatment. Aims. The study analysed retrospectively the therapeutic response in patients with ITP followed in our institution between 1996 and 2005. *Method*. A precise number of test was done to all thrombocytopenic patients. After the differential diagnosis a positive ITP diagnosis was established in 43 patients, 34 women and 9 men. They were treated commonly with various doses of corticosteroids. Other administered treatments included intravenous immunoglobulin (IVIg), splenectomy, vinca alkaloids, platelet concentrate and rituximab. We consider a sustained response a platelet count above 50,000/μL or above 30,000/μL without hemorrhages or only with minor purpura. A complete response was considered a platelet count above 150,000/μL after the discontinuation of therapy. *Results*. The mean age of the patients at diagnosis was 36,18±16,44years, (range, 9 and 74). The mean time from diagnosis was 26,69 months (0-288), with 19 new cases. The mean platelet count before treatment was 22,348±2,886/μL, with limits between 1,000 and 117,000. The initial treatment consisted of oral prednisone or methylprednisolone, 1-2 mg/kg/day. High-dose therapy consisted in 40mg dexamethasone by intravenous infusion (ivi) or 250-1000mg methylprednisolone ivi, both per day, for 4 consecutive days. Splenectomy was consider after 3 to 6 months in patients resistant to corticosteroids or earlier at patient demand. The response to corticosteroids, appreciated by the raise of platelets count, in the 40 patients treated this way, was obtained in 25 cases (62,5%), was partial in 5 cases and in 10 patients there were no response. The complete response to corticosteroids was sustained only in 6 cases (15%). In other 11 cases the response was sustained but the platelet count was well below 150,000 (total response rate 39,53%). In 10 cases splenectomy was done and 4 cases attained remission, in 4 cases the dose of prednisone needed decreased and in 2 cases no effect was observed. There was only one death (15 years after diagnosis) in our study (2,32%) and no severe infection in patients with splenectomy. *Conclusions.* The diagnosis of ITP covers a large spectrum of patients, with low mortality, but with important morbidity and treatment difficulties. Response to corticosteroids was as predicted. Splenectomy was curative in only 40% of patients previously resistant to corticosteroids. A complete response to corticosteroids was observed repeatedly in our single fatal case.

1061

TREATMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA: A SINGLE CENTER EXPERIENCE

P.R. Scalzulli,[1] M. Centra,[2] N. Dello Iacono,[2] M. Dell'Olio,[3] A.P. Falcone,[3] G. Fania,[2] G. Granatiero,[2] L. Melillo,[3] P. Musto,[3] M. Nobile,[3] M.R. Valvano,[3] G. Di Giorgio,[2] N. Cascavilla[3]

[1]*IRCCS 'Casa Sollievo della Sofferenza', San Giovanni Rotondo, Italy;* [2]*Transfusional Center, IRCCS 'CSS', San Giovanni Rotondo, Italy;* [3]*Hematology, IRCCS 'CSS', San Giovanni Rotondo, Italy*

Background. TTP is a severe syndrome, often with an subtle beginning, characterized by several clinical manifestations as thrombocytopenia, microangiopathic hemolytic anemia, fluttering neurological signs, renal failure and fever. These clinical manifestations are due to the formation of thrombi rich in platelets in the microcircle with consequent tissue ischemia. Pregnancy, infections (*E.Coli* and Entherohaemorragic 0157) and neoplasia may represent triggering factors. In some cases an important pathogenic role is represented by a constitutional or acquired (autoantibodies) deficiency of a metalloprotease, 13th member of ADAMTS (A Desintegrin And Metalloprotease with TromboSpondin type I domain) family. This metalloprotease deficiency affects the proteolytic degradation of VWF (Von Willebrand Factor) multimers inducing thrombocytic aggregation at the basis of microangiopathic thrombotic process. Fresh Frozen Plasma (FFP) therapy is the most efficient, inducing remission in 80% of patients, while there isn't agreement as yet on the efficiency of therapies with corticosteroids, antiaggregants, immunosoppressors and anti-CD20 (Rituximab) antibodies. *Aim of the Study*. In the present study we have evaluated in a retrospective way the therapeutic efficacy of plasma-exchange in patients with TTP that have been treated in our clinical division since March 1998 to January 2006. *Patients and Methods*. Thirteen patients (4 males and 9 females), mean age 42 years (14-62) were studied. They had at clinical presentation: 5/13 (38%) neurological symptoms, 2/13 (15%) acute renal failure, Mean PLTs 30x103μl (8-100), Mean Hb 8.0 gr/dL (6.0-10.0), Mean LDH 2541 U/dl (637-4000), Mean reticulocytes 7% (3-7). All patients immediately received therapy sessions with plasma-exchange (OCTAPLAS- KEDRION®) using KOBE SPECTRA® apparatus; mean sessions 7.8 (2-16); all patients received acetylsalicylic acid and 8/13 (61%) corticosteroids. *Results*. 10/13 (77%) patients showed a haematological response with a complete disappearance of neurological symptoms and renal failure; 3/13 (23%) died precociously cause of disease progression. PLTs number > 100×10^3 μl and Hb > 10 gr/dL was reached with an average of 5 (2-9) and 8 (4-19) plasma exchange sessions, respectively. 6/13 (46%) patients showed hypocalcemia, 4/13 (30%) hypokaliemia, 2/13 (15%) hypotension. None of them had fever, infections or PT alterations. 2/10 patients in remission had relapse and were recovered with new plasma-exchange sessions. One patient who relapsed for 5 times, was treated again with plasma-exchange, Vincristine and Cyclophosphamide, but without effect After the demonstration of *ADAMTS 13* activity reduction and anti *ADAMTS 13* antibodies level increase, 4 Rituximab courses administration allowed a complete remission lasting since 2 years. *Conclusions*. Our experience confirms that plasma-exchange treatment in TTP remains the most effective and safe in inducing remission of both haematological signs and related symptoms. The side effects linked to this treatment often are weak and immediately reversible in absence of mortality. Further studies are required in order to evaluate Rituximab therapy in TTP patients with recurrent relapses, with reduced ADAMTS 13 activity and/or anti ADAMS 13 antibodies increase.

1062

RITUXIMAB TREATMENT FOR REFRACTORY IMMUNE THROMBOCYTOPENIC PURPURA, HAEMOLYTIC ANEMIA AND EVANS SYNDROME

A. Müller-Soyano,[1] A.E. Soyano,[2] A. Soyano,[3] H. Goldstanj,[4] A. Urbáez,[4] M. Di Stefano[4]

[1]*Escuela de Medicina Luis Razetti (UCV), Caracas, Venezuela;* [2]*Clnica El vila, Caracas, Venezuela;* [3]*Inst. Venez. de Invest. Cientf. (IVIC), Caracas, Venezuela;* [4]*Hospital de Clnicas Caracas, Caracas, Venezuela*

Refractory chronic autoimmune diseases such as immune thrombocytopenic purpura (ITP), autoimmune haemolytic anemia (AIHA) and Evans syndrome are difficult to treat therapeutically. Recent studies have shown that Rituximab is useful in the treatment of these patients. Rituximab is an anti-CD20 monoclonal antibody which can deplete antibody-producing B-cells. The elimination of B cells is, in part, mediated through induction of complement activation and triggering of antibody-dependent cellular cytotoxicity. Rituximab has also been shown to directly activate intracellular pathways for apoptotic B cell death. In this paper 14 patients with refractory chronic immune diseases have been treated with Rituximab: nine (9) patients with chronic ITP, four (4) with recurrent AIHA and one (1) with Evans syndrome. Patients with chronic ITP for 5 to 21 years (four of the patients also with diagnosis of SLE), 5 to 75 years-old (3 children and 6 adults), with platelet counts < 40,000 /uL, antiplatelet antibodies, normal bone marrow cellularity and megakaryocyte count received Rituximab at a dose of 375 mg/m^2, once weekly for 4 weeks. Platelet response was characterized as complete (CR) if a count >150,000/uL was achieved and partial (PR) when the count was from 50,000 to 150,000/uL. Four adult patients (21-69 years-old) with autoimmune haemolytic anemia with positive direct Coombs test (IgG) and one patient (43 years-old) with Evans syndrome also received Rituximab. Haemoglobin response was characterized as complete if normal Hb and Hto for their sex and age was achieved and partial if their Hb increased at least two grams. Determination of CD4+ T cells and CD20+ B cells was done by flow cytometry with monoclonal antibodies. Results. Seven patients with ITP (six adults and one child, 77.7%) responded to Rituximab; two of them had been previously splenectomysed. Six patients had been in CR for one year to 16 months; four of these patients (all adults; 66.6%) relapsed one year after Rituximab (three of them were retreated with Rituximab without response). One adult patient had been in CR for only one month. One 9 years-old

child did not finish the treatment due to allergic reaction. Two other children did not respond. Three patients with AIHA has been in CR for 1 to 2.4 years and ongoing. One patient (21 years-old) had CR for only one month. The patient with Evans syndrome has been in CR for 14 months and ongoing. Therapy was well tolerated, except for an allergic reaction in two patients, and no infectious complications occurred. Steroids were withdrawn in patients in CR. The CD20+ B cell count decreased in most patients to less than 1% after Rituximab. Patients with refractory chronic immune cytopenias respond well to Rituximab, even after splenectomy. Rituximab may be consider before splenectomy in patients with high risk of complication, and it allows to withdraw steroids when the patient enter in CR.

1063

QUANTIFICATION OF SEMINAL THROMBOMODULIN

A. Lwaleed,[1] B. Lwaleed,[1] A. Goyal,[2] G. Delves,[2] R. Greenfield,[3] A. Cooper[2]

[1]*Southampton University Hospital NHSTrust, Southampton, United Kingdom;* [2]*University of Portsmouth, Portsmouth, United Kingdom;* [3]*American Diagnostica Inc, Stamford, CT, USA*

Backgrounds. Semen forms a gel-like-coagulum immediately after ejaculation, embracing the sperm. Subsequently semen liquefies spontaneously, after 5-20 min. The presence of fibrin degradations products, prothrombin fragments, and other active components of the plasma clotting system in seminal plasma have previously been reported. *Aim*: To investigate the presence of thrombomodulin in human semen. *Materials and Methods.* Using an Imubind® Thrombomodulin ELISA assay - seminal thrombomodulin antigen levels were measured in 37 semen specimens obtained from sub-fertile, normally fertile, fertile sperm donors and vasectomized subjects. *Results.* Thrombomodulin was quantifiable in human semen. The vasectomy group showed the lowest value. Slightly higher levels were seen for the normal, fertile sperm donors and the pooled normal semen parameters stratification group (derived from the World Health Organization [WHO] fertility criteria) compared to the infertile subjects. However, there were no significant differences between these groups when tested against each other. Seminal thrombomodulin levels showed negative association with total sperm concentration (density), sperm counts per ml, days of abstention, liquefaction time and semen volume. *Conclusion*: Our results establish the presence of thrombomodulin in human semen. Thus, provide further evidence for some involvement of the conventional haemostatic system in the coagulation and liquefaction properties of human semen.

1064

CLONAL PATTERNS OF HEMATOPOIETIC STEM CELLS IN PERIPHERAL BLOOD OF PERSONS ACCIDENTALLY EXPOSED TO HIGH DOSES OF RADIATION

Y.V. Olshanskaya,[1] L.A. Vodinskaya,[1] N.M. Nadezhina,[2] V.Y.U. Nugis,[2] E.V. Domracheva[1]

[1]*National Center for Hematology, Moscow, Russian Federation;* [2]*Institute of Biophysics, Moscow, Russian Federation*

Background. The polyclonality of hematopoiesis was revealed using individually marked hematopoietic stem cells (HSC) in mice, dogs and primates. The insertion site analysis of human hematopoietic cells engrafted in immune-deficient mice identified several individual clones that contributed to hematopoiesis. However, the relevance of these xenograft models to natural human hematopoiesis in vivo remains unclear. HSC of persons exposed to high doses of radiation bear stable chromosome aberrations during whole life. *Aims*. The dynamics of HSC functioning in vivo is poorly understood because of the difficulty in clonally tracking individual stem cells. The goal of this study was to investigate clonal contribution to hematopoiesis in humans. *Methods*. Stable chromosome aberrations in individual HSC were used to evaluate the fate of distinct human hemopoietic clones bearing unique chromosome markers in peripheral blood cells in persons after high-dose irradiation and consecutive hematological recovery. Clonal chromosome aberrations were evaluated in individual colonies in semisolid media developed from peripheral blood cells and in PHA-stimulated lymphocytes. Five healthy donors and 7 persons after *in vivo* exposure to 1,9-5,4Gy from 2 to 48 years before investigation were studied. There were no clonal chromosome aberrations in bone marrow cells, peripheral blood lymphocytes and individual colonies derived from healthy donors. Results. Chromosome analysis of individual colonies was performed in 5 cases of irradiated persons. Frequency of colonies with unique clonal markers varies from 0 to 100% depending on exposure doses. In patients 1 and 2 (exposure dose - 3,8 and 5,4Gy) colonies with different clonal markers were found, the same unique clonal chromosome markers were sometimes revealed in 2-3 colonies. During 2003-2005 years patient 2 was analyzed three times repeatedly and no colonies with the same aberration were found. Patient 3 (exposure dose - 3,6Gy, 20 years ago) was analyzed only once and all available colonies showed bearing the same marker looking as del(22q) or t(?;22) (translocation t(9;22) could not be excluded). In the other two patients (exposure dose - 1 and 2,3Gy) colonies with clonal chromosome markers were not found. In peripheral blood lymphocytes of 4 patients stable chromosome aberrations were found in 22-33% of cells depending on irradiation dose. In two of them 9-15% of aberrations were clonal. From 4 to 8 clones were found in each case, some of the clones were large and represented 2-3% of evaluated cells. The indication of stable chromosome aberrations in T-lymphocytes can be explained by both a direct radiation effect on long-living lymphocytes and indirect cell defects originating from HSC. *Summary/Conclusion*. Our preliminary results suggest that hematopoiesis in humans is polyclonal. The size of the clones, their longevity and kinetics will be the subject of further investigation.

1065

EXCELLENT STEM CELL MOBILIZATION USING ESCALATED BEACOPP IN HIGH-RISK PATIENTS WITH HODGKINS DISEASE

G. Kandler, M. Fillitz, M. Moestl, E. Schloegl, R. Reisner, E. Pittermann-Hoecker, T. Noesslinger, M. Pfeilstoecker

Hanusch Hopspital, Wien, Austria

Introduction: After intensive treatment regimens have been established, the survival rate for patients with advanced Hodgkin's disease is approximately 91% after five years and 13% of the patients have a relapse or have primary progressive disease (2%) within the first five years.For patients with relapse after conventional chemotherapy ± radiotherapy, however, there is a real chance of achieving remission again. Since it is often difficult to harvest autologous stem cells following an intensive pretreatment, our center embarks on the strategy to harvest autologous blood stem cells in high-risk patients, defined according to the risk stratification of the German Hodgkin's Study Group, already as part of the initial polychemotherapy. *Results.* Between 9/2003 and 2/2006, we analyzed the results of the stem cell harvest of 14 consecutive patients with Hodgkin's disease who were mobilized with the escalated BEACOPP regimen. There were 9 female and 5 male patients. Escalated BEACOPP was the primary therapy in twelve patients and a relapse was treated in two patients; the previous treatment was 4 or 6 cycles of the ABVD regime + involved field radiation. The twelve patients who did not receive previous treatment were classified as having Ann Arbor stage IIA/2 IIB/5, IIIB/3 and IVB/2 and most of them had a large mediastinal bulk as an additional risk factor. The two patients who did receive a previous treatment were classified as having an initial Ann Arbor stage IIA or IIB, without an additional risk factor. The stem cells were collected in 1 patient from cycle 2, in 9 patients from cycle 3 and in 4 patients from cycle 4 of the escalated BEACOPP regimen. A total of 13 patients received a standard dose of filgrastim, 5 µg/kg body weight s.c., from day 8 up to the last apheresis and 1 patient received pegfilgrastim 6mg s.c. All aphereses were performed using an Amicus cell separator™ (Baxter, MNC set, closed two-arm). 9 patients required only 1 apheresis and the remaining 5 patients required 2 aphereses. An apheresis result sufficient for a possible reinfusion could be achieved in all patients ($4.26 - 14.4 \times 10^6$ $CD34^+$ cells/kg/body weight, mean: 7.7). *Summary*. According to our experience, escalated BEACOPP regimen is very suitable for the harvesting of stem cells in high-risk patients with Hodgkin's disease even though they are receiving procarbazine. A sufficient quantity of stem cells can also be collected from pretreated patients. The stem cell mobilization can be integrated into the escalated BEACOPP regimen safely and without a delay in treatment and thus creates, already at an early stage, the precondition for a high-dose therapy, which might be required in high-risk patients

1066

MARROW CELLS CULTURED IN MSC MEDIUM EXPAND TO CD73, CD90 AND CD105 CELLS OF FIBROBLAST-LIKE MORPHOLOGY

A.L. Lange, D.D. Dlubek, D.D. Drabczak-Skrzypek, B.K. Bogunia-Kubik, J.E. Jaskula

Institute of Immunology, Wroclaw, Poland

Backgrounds. Recent literature data suggest that in the marrow reside progenitors with a potential to regenerate not only hematopoietic sys-

tem but also different damaged tissues. Cells of the latter potential may belong to Mesenchymal Stem Cell population (MSC). *Aim of study*: The present study was undertaken to: (i) identify cells with MSC characteristics, (ii) enrich marrow cells in MSC, (iii) propagate MSC *in vitro*, (iv) characterize cultured cells. *Methods.* Marrow cells obtained from 13 individuals (patients suffering from critical leg ischaemia receiving autologous marrow cells to promote angiogenesis) were processed as follow: (i) fresh BM cells and their mononuclear cells subfraction - BM MNC (Cobe Spectra 6.0) were labeled for CD45, CD34, CD73, CD90, CD105 and CXCR4, (ii) BM MNC populations were enriched in MSC with the use of negative selection depleting cells having: Glycoforin A, CD3, CD14, CD19, CD66b, CD38, (iii) BM, BM MNC and BM MNC MSC+ were cultured for 14 days for colony forming-fibroblast unit (CFU-F) enumeration, (iv) cultures of BM MNC and BM MNC MSC+ were continued until >90% confluence of fibroblast-like cells then passaged for optimal growth. Proportions of cells were used for molecular biology study (quantitative analysis of SDF-1 and CXCR4 transcripts using Real Time PCR assay). *Results.* (i) CD45-CD34-, CD45-CD34-CD73+, CD45-CD34-CD90+, CD45-CD34-CD105+ were present in BM vs BM MNC vs BM MNC MSC+ in proportions as follows (median value): 9.4, 0.04, 0.05, 1.28 vs 11.01, 0.66, 0.89, 22.40 vs 59.0, 0.68, 1.09, 32.41 respectively (ii) BM MNC MSC+ had higher proportions of CFU-F as compared to BM MNC and BM (106,5 vs 21,5 vs 2,0 CFU-F/106 cells, median value), (iii) all BM MNC MSC+ cells were strongly CXCR4 positive and had high levels of CXCR4 gene transcripts, (iv) during 6'8 weeks cultures cells having MSC phenotypic characteristics expanded from 5.5 to 11.5 folds and all expanded cells were positive for CD105, CD73 and CD90 and had fibroblast-like cells morphology, (v) cultured cells showed expression of SDF-1 and CXCR4 genes, however, the latter gene transcripts level decreased gradually during the culture. This trend was seen in 11 out of 13 evaluated cultures. *Summary*. Cells of MSC characteristics are enriched in BM MNC population, expand several-folds under MSC culture conditions to cell population of fibroblast-like cells morphology but have lower levels of CXCR4 transcripts as compared to starting the populations.

1067

HUMAN BONE MARROW ADIPOCYTES AND HEMATOPOIESIS: FROM UNILOCULAR FAT CELLS TO FIBROBLAST-LIKE FAT CELLS AND THEIR RELATION WITH CD34+ PROGENITORS DIFFERENTIATION IN THE ABSENCE OF EXOGENOUS CYTOKINES

J. Hernandez Rivas

Hematologia, Salamanca, Spain

Backgrounds. The bone marrow microenvironment plays a critical role in regulating the growth and differentiation of hematopoietic cells. Both growth factors and cytokines, as well as direct cell-cell contacts, participate in these processes. Fat cells are heterogeneously present in the bone marrow and replace hematopoietic cells in bone marrow failure disorders. Thus, it is usually admitted that they play a passive role in hematopoiesis. *Aims.* In this work we have tested the hypothesis that adipocytes could play an active role in hematopoiesis. *Methods.* Adipocytes isolation, cell culture,RT-PCR, optic cytology, electronic microscopy,immunophenotypic analysis (FACS, confocal) and ELISA. *Results.* cocultures of FLFC and CD34+ positive cells, induce CD34+ differentiation essentially into macrophages (Mo) and dendritic cells (DC). Likewise, by RT-PCR and ELISA analysis, these cells constitutively produce SCF, M-CSF and GM-CSF. In contrast, granulopoiesis was poorly represented and erythopoiesis was totally inhibited even in the presence of high dose of Erythropoietin (2U/mL). FLFC establish cell-cell contacts with Mo and DC, but this contact is however not critical since DC and Mo are obtained in transwell coculture. In contrast, in transwell experiments erythropoiesis and granulopoiesis were restored. *Summary/Conclusion*: Our data suggest that adipocytes have an active role in hematopoiesis. They may induce CD34+ cells differentiation towards Mo and DC, and inhibit through cell-cell contact, erythropoiesis and granulopoiesis. Our data may provide a new role for adipocytes in vivo, and may actively participate at the pathophysiology of bone marrow failure disorders. It remains to determine by which mechanisms FLFC operate in this process, to define new therapeutic strategies able to target FLFC.

1068

HEMOLYTIC CRISIS DUE TO RASBURICASE IN A PATIENT WITH PREVIOUSLY UNKNOWN GLUCOSE-6-DEHYDROGENASE DEFICIENCY

M.A. Ardaiz, J.M. Arguiñano, M.C. Mateos, M.C. Montoya, M.A. Goñi, A.M. Redondo, M.J. Paloma, I. Ezpeleta, M.A. Labaca, F.J. Oyarzabal

Hospital Virgen del Camino, Pamplona, Spain

Glucose 6 phosphate dehydrogenase deficiency is a widespread congenital eritroenzymopathy, more frequent in individuals of African and Mediterranean ethnical origin. Hemolysis is arised by infections, certain foods and several drugs. Avoiding such precipitants is the only therapeutic measure. Rasburicase is a recombinant enzyme with urate oxydase activity employed for tumour lysis syndrome prophilaxys and therapy. It is formally contraindicated in patients with decreased glucose 6 phosphate activity due to its oxidant activity capable of inducing haemolytic crises. We report the case of a 30-year-old male patient of African origin diagnosed of diffuse large cell B lymphoma stage IIIE B affecting stomach and presenting as a massive gastric hemorrhage. At diagnosis bilirrubin was within normal limits and LDH was 1086 U/L. Anemia of 9,1 g/dL haemoglobin prompted red blood cell transfusion, then rising to 10,7 g/dL. Gastric hemorrhage precluded oral route for drugs, and alopurinol is not available in the intravenous form in our institution, so we started prophylaxis of tumour lysis syndrome with rasburicase once therapy with rituximab and CHOP was started. Once three doses of rasburicase had been administered the patient complained of dark urine, which turned to be black. In analysis anemia worsened suddenly falling to 6,9 g/dL; blood smears showed schistocytes and eccentrocytes not previously noticed. In chemistry bilirrubin of 3,8 mg/dL, LDH of 4551 U/L and haptoglobin less than 6,5 mg/dL were the most important findings. A diagnosis of haemolytic crisis was made, rasburicase stopped and vigorous hydration with alkalinization instituted, as well as folinic acid supplementation. Evolution was favourable, requiring transfusion of five red blood cell units. The patient was questioned for past haemolytic crises with negative results, since no episodes of dark urines were reported. Decreased glucose 6 phosphate dehydrogenase level despite previous blood cell transfusion was found, leading to the diagnosis of enzyme deficiency. Thus rasburicase caused the haemolytic crisis in this patient because of a previous enzyme deficiency. No haemolytic crises have appeared in subsequent courses of chemotherapy and rituximab. Rasburicase is formally contraindicated in glucose 6 phosphate dehydrogenase deficiency. This defiency was suspected once hemolysis had started since no previous history of haemolytic crises had been reported. In patients of certain ethnic origin (African , Mediterranean) levels of glucose 6 phosphate dehydrogenase should be determined before administering treatment with rasburicase even without a previous history of haemolitic crises.

1070

LOSS OF CASPASE-8 EXPRESSION DOES NOT CORRELATE WITH MYCN AMPLIFICATION, AGGRESSIVE DISEASE OR PROGNOSIS IN NEUROBLASTOMA

S. Fulda,[1] C. Poremba,[2] B. Berwanger,[3] S. Haecker,[1] M. Eilers,[3] H. Christiansen,[4] B. Hero,[4] K.M. Debatin[1]

[1]*University Children's Hospital, Ulm, Germany;* [2]*Heinrich-Heine-University, Dusseldorf, Germany;* [3]*IMT, Marburg, Germany;* [4]*Children's Hospital, Marburg, Germany*

Inactivation of caspase-8 because of aberrant gene methylation has been associated with amplification of the MYCN oncogene and aggressive disease in neuroblastoma suggesting that caspase-8 may function as tumor suppressor. However, the prognostic impact of caspase-8 in neuroblastoma has remained obscure. Therefore, we investigated caspase-8 expression and its correlation with established prognostic markers and survival outcome in a large cohort of neuroblastoma patients. Here, we report that loss of caspase-8 protein expression occurs in the majority (75%) of neuroblastoma and is not restricted to advanced disease stages. Surprisingly, no correlation was observed between caspase-8 expression and MYCN amplification. Similarly, ectopic expression of MYCN or antisense-mediated downregulation of MYCN had no effect on caspase-8 expression in neuroblastoma cell lines. Also, caspase-8 expression did not correlate with other parameters of high-risk disease, e.g. 1p36 aberrations, disease stage, age at diagnosis or tumor histology. Most importantly, loss of caspase-8 protein had no impact on event-free or overall survival in the overall study population or in distinct subgroups of patients. By revealing no correlation between caspase-8 expression

and MYCN amplification or other established parameters of aggressive disease, our findings in a large cohort of neuroblastoma patients demonstrate that inactivation of caspase-8 is not a characteristic feature of aggressive neuroblastoma. Thus, our study provides novel insight into the biology of this tumor, which may have important clinical implications.

1071

SENSITIZATION OF GLIOBLASTOMA CELLS FOR DEATH RECEPTOR- OR ANTICANCER DRUG-INDUCED APOPTPSIS BY PI3K INHIBITION

S. Fulda, D. Opel, M.A. Westhoff, K.M. Debatin

University Children's Hospital, Ulm, Germany

Activation of the PI3K/Akt/mTOR pathway has recently been reported to correlate with increasing tumour grade, decreased apoptosis and adverse clinical outcome in human malignant glioma in vivo. However, the therapeutic potential of targeting the PI3K/Akt/mTOR cascade by kinase inhibitors for apoptosis sensitization of malignant glioma has not yet been investigated in detail. Here, we report that inhibition of PI3K by LY294002 significantly sensitized glioblastoma cells for death-inducing ligands (TRAIL, agonistic anti-CD95 antibodies) as well as for different anticancer drugs (Doxorubicin, Taxol, Vincristin). In contrast to PI3K inhibition, blockade of mTOR by RAD001 (everolimus) or of MEK by UO126 did not significantly alter the sensitivity of glioblastoma cells for TRAIL- or Doxorubicin-induced apoptosis. Analysis of apoptosis pathways revealed that inhibition of PI3K resulted in downregulation of antiapoptotic proteins such as FLIPs, XIAP, cIAP2 and survivin and cooperated with TRAIL or Doxorubicin to trigger loss of mitochondrial membrane potential, release of cytochrome c from mitochondria and full activation of the caspase cascade. Inhibition of caspases by the broad range caspase inhibitor zVAD.fmk completely abolished apoptosis in response to combined treatment with LY294002 and TRAIL or Doxorubicin, indicating that apoptosis occurred in a caspase-dependent manner. By demonstrating that inhibition of PI3K significantly enhanced both death receptor- and anticancer drug-induced apoptosis in glioblastoma cells, our findings have important implications for the development of novel treatment strategies in glioma therapy. Thus, PI3K inhibitors represent a promising approach to enhance the antitumor activity of TRAIL or chemotherapy in glioblastoma.

1072

HBVAR-XPRBASE: A COMPREHENSIVE ONLINE REPOSITORY OF EXPERIMENTAL PROTOCOLS TO SCREEN FOR HUMAN GLOBIN GENE SEQUENCE VARIATIONS

P. Kollia,[1] S. Van Baal,[2] M. Samara,[1] G. Patrinos[2]

[1]*University of Thessalia, Larissa, Greece;* [2]*Erasmus MC, Faculty of Medicine, Rotterdam, Netherlands*

Backgrounds. Hemoglobinopathies, resulting from mutations in the α- or β-like globin gene clusters, are the most common inherited disorders in humans, with approximately 7% of the world population being carriers of a globin gene mutation. Single nucleotide substitutions can lead to amino acid replacements that cause hemolytic anemias, such as sickle cell disease, or hemoglobins that are unstable or have altered oxygen affinity. Molecular defects in either regulatory or coding regions of the human α-, β-, or δ-globin genes can minimally or drastically reduce their expression, leading to α-, β- or δ-thalassemia, respectively. Other sequence changes have little or no effect on hemoglobin function, but are useful polymorphisms for genetic studies. A plethora of mutation detection methods are currently available for human globin gene mutation screening (*Patrinos et al., 2005*). Recently, a locus-specific database, HbVar (*http://globin.cse.psu.edu, Hardison et al., 2002*), has been developed by a multi-center academic effort, in order to provide globin research community with (i) up-to-date and high quality information on the genomic sequence changes leading to hemoglobin variants and hemoglobinopathies, (ii) globin gene mutation frequencies in various populations (*Patrinos et al., 2004*) and (iii) the option to combine information on hemoglobin variants and thalassemia mutations with a wide spectrum of genomic data. *Aims.* The construction of HbVar-XPRbase (*http://www.goldenhelix.org/xprbase*), an electronic database aiming at collecting to a single website all the experimental protocols available for mutation screening in the human globin genes. Database contents: HbVar-XPRbase is a curated database, which includes a concise listing of the available globin gene mutation screening strategies. HbVar-XPRbase is a flat-file database and operates under PHP. The available experimental protocols to screen for the different human globin gene mutations and/or polymorphisms have been extracted from the published literature, re-constructed to be more concise and comprehensive and made available for the users to query upon. Information in this database is stored in such way that the user can formulate different queries, for example for the available DGGE protocols for mutation screening in all globin genes, or for the mutation detection technologies only for the δ-globin gene. In addition, links to specific globin gene mutations, stored in HbVar, re-direct the user to the mutation detection strategy, by which these mutations have been identified. Emphasis has been given so that the protocols stored within HbVar-XPRbase describe a general mutation detection strategy, spanning across the entire genomic region of a particular globin gene. *Conclusions.* HbVar-XPRbase provides a comprehensive collection of human globin gene mutation screening protocols, allowing researchers and diagnostic laboratories to easily choose from a single website, those protocols which suit better to their needs.

1073

ALTERATIONS OF GENE EXPRESSION IN PTH (1-34) TREATED LONG-TERM BONE MARROW CULTURES FROM PATIENTS WITH APLASTIC ANEMIA

T.V. Petrova, D.A. Svinareva, I.N. Nifontova, M.A. Vinogradova, E.A. Michaylova, N.J. Drize

National Hematology Research Centre, Moscow, Russian Federation

Background. Pathophysiology of aplastic anemia (AA) is not well understood. An impairment of regulation of the hematopoietic potential could be caused by abnormal interaction between AA hematopoietic stem cells and their microenvironment. It is possible to establish stromal layer from AA patients *in vitro*, but it fails to support healthy hematopoietic stem cells, suggesting a defect in the marrow microenvironment of the patients. Osteoblastic cells were shown to be one of the hematopoietic stem cell niche participants. Administration of PTH leads to increased generation of osteoblasts as well as enhanced osteoblast function. An increase in the number of stem cells was observed in animals after PTH injection, and survival after bone marrow transplantation was markedly improved. PTH treatment of long-term bone marrow culture improved adhesion of stem cells to stromal layers and maintenance of hematopoietic precursor cells. *Aims*. The aim of this study was to find out if PTH treatment could cause any alterations in expression of some genes in adherent cell layers (ACL) of cultures from AA patients. *Methods*. Long-term bone marrow cultures were established from 19 patients with aplastic anemia and 24 donors as a control group. PTH was added for 3 and 6 weeks of cultivation in concentration 10-8, 5×10^{-8} and 10^{-7} M once a week while changing half of the media. To characterize alterations in the expression of several genes in ACLs after PTH treatment semi-quantitative analysis of RT-PCR products was performed using PhosphoImager Cyclone, Packard Bell (USA) after Southern blot hybridization with appropriate sequences. The expression level of β-actin was used as a normalization factor. *Results*. Osteblastic cells activated by PTH produced high levels of the Notch ligand Jagged 1. Expression level of Jagged 1 increased insignificantly in donors' ACLs after PTH treatment and did not change in AA patients' ACLs. In ACLs of both types the expression level of Notch was stable and independent of PTH treatment or duration of cultivation. Expression level of Bmi 1 and Ang 1 genes taking part in regulation of HSC proliferation did not change in PTH-treated cultures from AA patients. Moreover, after 3 weeks in culture expression of Ang 1 was 3-fold lower in these cultures compared with donor ones. In donor ACLs PTH administration caused 3-fold increasing of Bmi 1 expression. Expression levels of cell adhesion molecules VCAM 1 and ICAM 1 in ACLs of both donors and AA patients were not sensitive to PTH treatment while in donor ACLs expression of ICAM increased significantly during cultivation. VEGF expression increased in donor ACLs after both PTH treatment and cultivation, whereas no changes in its expression were observed in AA patients ACLs. *Summary/Conclusion*. Stromal cells from patients with AA are not sensitive to PTH treatment. It may happen due to absence of activation of osteoblastic cells in their microenvironment after PTH administration and may point to pathology of these cells.

1074

ACUTE MYELOID LEUKEMIA: OUTCOME OF RELAPSE FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION COMMENTS ON 46 CASES

A.G. Agathocleous,[1] M. Bouzani,[2] G. Kourti,[2] V. Delistrati,[2] Z. Poulopoulou,[2] I. Bika,[2] I. Baltadakis,[2] I. Apostolidis,[2] D. Karakasis,[2] N. Harhalakis,[2] E. Nikiforakis[2]

[1]*'Evaggelismos' General Hospital, Athens, Greece;* [1]*'Evaggelismos - General Hospital, Athens, Greece*

Acute myeloid leukemia (AML) patients relapsing after allogeneic stem cell transplantation (allo-Tx) have a very poor prognosis. Discontinuation of immunosuppresion and donor lymphocyte infusion exhibit activity against leukemic cells. However, long term outcome is disappointing. Patients' selection for salvage treatment is very important. Forty-six consecutive AML patients relapsed after allo-Tx between 9/1992 and 7/2005. Age at transplantation ranged between 15 and 60 years (median, 36). Thirteen patients were in early, 14 in intermediate and 19 in advanced stage at the time of transplantation. Donors were HLA identical siblings (n=34), mismatched related (n=6) and matched unrelated (n=6). In all but one cases ablative conditioning was used. In all but one (haploidentical sib with 3 HLA Ag m/m), cases T-cell repleted grafts were given. Relapse occurred 1 to 90 months after transplantation (median, 5). Eight patients had an early relapse (< 30% blasts in BM), and two other had exclusively extramedullary relapse. Salvage therapy was introduced in 30 of the 46 patients and included: Combined chemotherapy (n=5), donor lymphocyte infusion (n=5), combined chemotherapy or serotherapy plus donor lymphocytes or haemopoietic cells (n=11), high dose chemotherapy plus haemopoietic cells (n=9). One patient suffering from acute promyelocytic leukemia was treated with As2O3 among other modalities. Eleven patients died due to therapy related toxicity (37%). Complete remission was achieved in 15 patients (50% of the patients receiving salvage therapy and 33% of the total relapsed patients). Four patients are alive and in CR for 3, 22, 40, and 53 months after relapse. In two out of these four patients acute and subsequently progressive chronic graft versus host disease developed for the first time after relapse. Overall survival for all patients is 12.7% at 4 years. Among eight patients with early relapse, 3 are alive and in CR for 3, 22, and 53 months. Both patients with extramedullary relapse are alive but still with relapsing extramedullary disease for 14 and 99 months. In conclusion, the very poor prognosis of relapse after allo-Tx in AML is confirmed. Even in selected patients salvage therapy is accompanied with high mortality rate. Nevertheless, it must be emphasized that in a small proportion of patients long and disease-free survival is achieved. A possible survival advantage of patients with early relapse (i.e. ≥ 30% blasts in BM) points out to the importance of close follow-up after Tx. Long survival (even not disease-free) of patients with extramedullary relapse is remarkable and the involved mechanisms deserve further studies.

1075

USE OF FROZEN EMBRYOS FOLLOWING STEM CELL TRASPLANTATION FOR LEUKEMIA: A SINGLE CENTRE EXPERIENCE.

N. Salooja, J.F. Apperley, S. Duffy, R. Margara, N. Reddy

Hammersmith Hospital, London, United Kingdom

Infertility is common following stem cell transplantation (SCT) for leukaemia. Options for parenthood need to be considered before high dose chemotherapy and total body irradiation are given and include cryopreservation of fertilised embryos, oocytes (unfertilised mature eggs) or ovarian tissue. The pregnancy rate per embryo transfer in otherwise healthy women is approximately 25% with a take home baby rate of approximately 16-18%. The success rate using frozen embryos in women who have received chemoradiotherapy as treatment for cancer is not known. There are theoretical concerns that success will be lower in these patients compared to the normal population because of the effects of chemoradiotherapy on the uterus. Pre-transplant chemotherapy or disease may also have deleterious effects on the female reproductive system. At the IVF Unit, Hammersmith Hospital, 6 women with an underlying diagnosis of CML have attempted pregnancy using embryos cryopreserved prior to SCT.

Table 1. Outcome of frozen embryo transfer.

	Treatment cycles	*Pregnant/ not pregnant (NP)*	*Outcome*
Patient 1	n=3	P	miscarriage
Patient 2	n=1	P	Live birth
Patient 3	n=1	NP	–
Patient 4	n=2	NP	–
Patient 5	n=2	P	miscarriage
		P	Live birth
Patient 6	n=2	NP	–

Of these 6 women, 5 had allogeneic SCT with conditioning which included total body irradiation and one had an autologous SCT conditioned with high dose busulphan only (patient 4). The results are tabulated below. Eleven treatment cycles using cryopreserved embryos in 6 women led to 4 pregnancies in 3 women. Of these, two pregnancies have been successful. There have been two miscarriages, one (patient 1) at 12-13 weeks gestation and the second (patient 5) at 7 weeks gestation. We conclude that the success rate using frozen embryos to achieve pregnancy following SCT is low. Despite the low pregnancy and low take-home baby rate, cryopreservation of embryos prior to SCT remains the best option for women likely to develop treatment-related ovarian failure and who wish to have their own genetic offspring following SCT.

1076

RETROSPECTIVE STUDY OF TRANSPLANTATIONS WITH CRYOPRESERVED ALLOGENEIC BONE MARROW OR PBPC

M.C.N. Ngirabacu, N. Meuleman, I. Ahmad, J. Bennani, L. Ysebrant, R. Leroy, P. Huynh, P. Martiat, D. Bron

Institut Jules Bordet, BRUSSELS, Belgium

Backgrounds. Cryopreservation of haematopoietic stem cell is regularly used in autologous stem cell transplantation. Its indication in allogeneic peripheral blood stem cell (alloSCT) or marrow transplantation is limited. *Patients and Methods.* We describe here 45 patients transplanted with cryopreserved bone marrow or Peripheral blood progenitor cells (PBPC) in our institution between 1999 and 2005. Median age at the time of transplantation was 41 (20-63) years. 29 patients underwent conventional alloSCT (16 HLA identical sibling and 3 Matched Unrelated Donors) and 16 benefited from non-myeloablative conditioning SCT (13 familial donors and 3 unrelated donors). The outcome of these patients was compared with historical control groups in our center. *Results.* In non-myeloablative conditioning alloSCT (NMSCT) group, median time to recover (WBC>1000/μL, ANC>500/μL, platelets >50000/μL) was 11 days. In the conventional alloSCT group, engraftment occurred on day 15 for WBC and on day 14 for ANC and platelets. Comparison with historical control groups in our institution failed to show a significant difference (median time for engraftment: 9 days for NMSCT and 17 days for conventional alloSCT). On day 100, overall survival was 67%. Immunological reconstitution ($CD4^+$ cells> 200/μL) was assessed after 6 and 12 months. Six months after allograft, none of the patients had reached more than 300 $CD4^+$ cells/μL, but in 3/4 patients, $CD4^+$ T cells were superior to 200/μL. At 1 year, immunological reconstitution was assessed in 12 patients. 58% of them had reached 200 CD4/μL. These results are very similar to the previous observations in our center. Conclusion: Altough the numbers are small, we suggest that the use of frozen stem cell in allogeneic bone marrow transplantation does affect neither engraftment, nor day100 survival, and thus, could be safely used.

1077

HEPATITIS B VIRUS REVERSE SEROCONVERSION IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

C. Vener,[1] D. Soligo,[1] M. Viganò,[2] P. Lampertico,[2] M. Colombo,[2] F. Lanfranchi,[1] G. Lambertenghi Deliliers[1]

[1]*Ematologia Centro Trapianti di Midollo, Milano, Italy;* [2]*Gastroenterologia, IRCCS, Milano, Italy*

Background and Aims. We conducted a retrospective study to define the risk factors and the clinical impact of reverse HBsAg seroconversion in HBsAg negative/anti-HBc positive patients after allogeneic hematopoietic stem cell transplantation (HSCT). *Patients and Methods*. We reviewed the Bone Marrow Transplant database from May 1998 to May 2005. Complete serological screening for HBV status in donors and recipients was available, before transplantation, in 207 out of 231 patients who underwent allogeneic HSCT due to onco-hematological diseases. Fifty patients (24%) were HBsAg negative/anti-HBc positive; ten patients died prior to day 100 after HSCT and could not be evaluated for long-term follow-up, forty patients were finally included in this study. The conditioning regimen consisted of chemotherapy ± total body irradiation. Graft-versus-host disease (GVHD) prophylaxis included standard-dose cyclosporin-A ± methotrexate or mofetil mycophenolate. Clinical and laboratory examinations were performed weekly for the first two months, monthly, for the rest of the first year and every six months thereafter. *Results.* During 37 months (range:4-96) post-HSCT, 6 (15%) patients showed reverse seroconversion, 12 months (range 7-32) after HSCT. All these 6 patients developed HBeAg-positive chronic hepatitis

B with >7 log_{10} copies/mL HBV DNA and 1-36 fold (median 4) ALT increase. Two patients developed jaundice but none developed clinical decompensation, no patients required hospitalisation. Two patients were treated with lamivudine 100 mg/daily; one patient's ALT levels and serum HBV-DNA progressively declined whereas the other patient developed lamivudine-resistance, requiring additional treatment with adefovir dipivoxil. Three out of 4 untreated patients maintained persistently high levels of serum HBV-DNA and abnormal ALT throughout the study period, whereas one lost HBsAg and developed anti-HBs. Reverse HBsAg seroconversion occurred in 4 out of 22 (18%) patients who developed chronic graft-versus-host disease (cGVHD) as compared to 2/18 (11%) who did not. Conclusion. The high rate of HBV reverse seroconversion and the development of chronic hepatitis B in all these patients, call for a prophylactic anti-HBV treatment in all HBsAg negative/anti-HBc positive patients undergoing HSCT.

1078

ANGIOGENIN LEVELS IN PATIENTS WITH POLYCYTHEMIA VERA

S. Theodoridou,[1] T. Vyzantiadis,[2] I. Venizelos,[1] S. Vakalopoulou[1], E. Mandala,[1] V. Perifanis,[1] E. Leukou,[1] I. Klonizakis,[1] V. Garipidou[1]

[1]*Hippokration Hospital, Thessaloniki, Greece;* [2]*1st Dep. of Microbiology, Medical School, Thessaloniki, Greece*

Angiogenin is a protein with a potent function in angiogenesis, which circulates in human serum and is secreted by haematopoietic cells, endothelial cells, vascular smooth cells and fibroblasts. Its serum levels are increased in patients with solid tumors, acute myeloid leukemia, myelodysplastic syndromes, chronic myeloid leukemia and essential thrombocytosis. No data are available about angiogenin levels in patients with polycythaemia vera. In this study we aimed to evaluate the levels of angiogenin in serum of patients suffering of polycythaemia vera and examine any possible correlation with bone marrow microvascular density (MVD) detected by CD34 count on bone marrow trephines. A total of 29 patients with PV (14 males and 15 females) with a mean age of 57 ± 15,4 (m ± SD) years (range 24-81) were included. The control group consisted of 16 healthy subjects (8 males and 8 females) with a mean age of 55,9 ± 6,7 years (range 46-71). Serum levels of angiogenin were measured by a commercial quantitative sandwich enzyme immunoassay. In twenty four of them we estimated the MVD in bone marrow samples immunostained with anti-CD34 monoclonal antibody by counting the number of vessels per 400x high power field (HPF) using light microscopy. Serum angiogenin concentrations were found to be significantly higher in polycythaemic patients than in the control group (434±145 pg/mL and 340±94 pg/mL, respectively, p=0,037). In the patient and in the control group we found no statistically significant correlation between serum angiogenin levels and platelet counts, haemoglobin, WBC counts and age. No difference was found between patients angiogenin levels on different therapeutic regimens. The microvessel density of the bone marrow of the 24 polycythaemic patients was found to be 7,1±4,1 vessels per HPF and significantly elevated in comparison to normal bone marrow specimens (n=10, MVD: 2,0±0,6, p=0,01). Interestingly a negative correlation was found between serum angiogenin levels and the microvessel density of the bone marrow (r=-0,49, p=0,033). In the present study, serum angiogenin levels were found to be significantly increased in patients with PV in comparison to the control group. To our knowledge there is evidence of pronounced angiogenesis in PV by few reports on other angiogenic factors as vascular endothelial growth factor and basic fibroblast growth factor. In addition the current study demonstrated increased MVD in comparison to healthy control group indicating augmented angiogenetic procedure. The observation that MVD is negatively correlated with angiogenin serum levels, although at first sight unexpected, could be explained by the hypothesis that in patients group the less bone marrow vascularity acts modulating the increase of angiogenin production which in its turn enhances the bone marrow vascular expansion. It is emphasized that this observation should be confirmed by other studies as well. The exact contribution of angiogenin in the pathophysiology of PV and its prognostic significance as disease activity marker deserves further research.

1079

HYPERHOMOCYSTEINAEMIA IS ONE OF THE FACTORS OF THROMBOTIC COMPLICATIONS DEVELOPMENT FOR PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

M.A. Sokolova, N.V. Tsvetaeva, A.G. Turkina, A.A. Levina, A.B. Sudarikov, E.A. Romanova, S.A. Vasiliev, N.D. Khoroshko

Hematology Research Centre RAMS, Moscow, Russian Federation

Myeloproliferative disorders (MPD), such as essential thrombocythemia (ET), polycythaemia vera (PV) and idiopathic myelofibrosis (IMF), characterised by clonal proliferation of haemopoietic stem cells, have an elevated risk of arterial and venous thromboembolic complications. *The aim*. Since hyperhomocysteinaemia (HHC) is a risk factor of vascular complications, we had investigated the frequency of HHC in these diseases. We had analysed the possible relationship between the elevated level of homocysteine (HC) in blood serum and vascular complications, and the prevalence of the methylenetetrahydrofolatreductase (MTHFR) enzyme mutation and the effect of this enzyme on HC level in blood. *Materials and results*. We analysed 61 patients: 39 patients with MPD with thrombotic episodes in medical history and without them, and 22 non-hematological patients with thrombotic episodes in medical history. Among 39 patients (22 females and 17 males, mean age 41 years, range17- 63 years) with ET (n=17), PV (n = 8), IMF (n = 14) the mean levels in blood serum were significantly higher (19±1,7 μmol/L) in comparison with 40 donors in control group (12±1,3 μmol/L, p<0,00002). In the group with thrombotic episodes the mean level in the patients with IMF was much higher (26±4,7 μmol/L), than for patients with ET (20±3,0 μmol/L) and PV (23±8,9 μmol/L), p<0,002. Despite of different frequency of alleles 677 of *MTHFR* gene in patients with MPD, HHC was observed with the same frequency in all groups, which was different from the situation in healthy people. We did not find out significant differences in the prevalence of genotypes (heterozygous, homozygous) in *MTHFR* gene in patients with MPD and donors: 72% (8/11) with thrombosis and 42% (8/19) without thrombosis, and accordingly 60% (24/40) in donors population. We did not find out a significant relationship between *MTHFR* genotype and the rate of thrombotic complications. For MPD patients with normal and elevated HC concentration in blood serum it was shown, that factor VIII level was higher in HHC, than in patients with normal HC level (222±26,5% and 116±20%, p=0,002). The same was found for von Willebrand factor (202±15,6, 120±14,6%, p<0,003). Comparing the data on ADP induced platelet aggregation in patients with thromboses, it was shown that the activation of the later was higher in HHC against normal data of blood HC (77±15,7% and 47±12,1%, accordingly, p<0,001). Regressional analyses showed that only HHC has a statistically significant influence on thrombotic complications rate for MPD patients (p=0,004). We consider that the lowering of HC plasma levels together with vitamin therapy were the more expressed, the higher was its baseline level. *Conclusion*. The relationship between HHC levels and thromboses in the patients with MPD was shown. We suppose that different stages of proliferative diseases may influence the HC levels in patients with MPD. Homocystein is a highly significant, independent risk factor for thrombotic complications. Therefore it is necessary to discover and treat HHC.

1080

HYPEREHOMOCYSTEINAEMIA IS ONE OF THE FACTORS OFF THROMBOTIC COMPLICATIONS DEVELOPMENT FOR PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

M.A. Sokolova,[1] N.D. Khoroshko,[2] N.V. Tsvetaeva,[2] A.G. Turkina,[1] A.A. Levina,[1] A.B. Sudarikov,[1] E.A. Romanova,[1] E.A. Orel[1], V.E. Rudakova[1]

[1]*RAMS, Moscow, Russian Federation;* [2]*Nina, Moscow, Russian Federation*

Myeloproliferative disorders (MPD), such as essential thrombocythaemia (ET), polycythemia vera (PV) and idiopathic myelofibrosis (IMF), characterised by clonal proliferation of hemopoietic stem cells, have an elevated risk of arterial and venous thromboembolic complications. *The aim*. Since hyperhomocysteinaemia (HHC) is a risk factor of vascular complications, we had investigated the frequency of HHC in these diseases. We had analysed the possible relationship between the elevated level of homocysteine (HC) in blood serum and vascular complications, and the prevalence of the methylenetetrahydrofolatreductase (MTHFR) enzyme mutation and the effect of this enzyme on HC level in blood. *Materials and results*. We analysed 61 patients: 39 patients with MPD with thrombotic episodes in medical history and without them, and 22 non-hematological patients with thrombotic episodes in medical history. Among 39 patients (22 females and 17 males, mean age 41 years,

range17- 63 years) with ET (n = 17), PV (n = 8), IMF (n=14) the mean levels in blood serum were significantly higher (19±1,7 μmol/L) in comparison with 40 donors in control group (12±1,3 μmol/L, p<0,00002). In the group with thrombotic episodes the mean level in the patients with IMF was much higher (26±4,7 μmol/L), than for patients with ET (20±3,0 micromol/L) and PV (23±8,9 μmol/L), p<0,002. Despite of different frequency of alleles 677 of *MTHFR* gene in patients with MPD, HHC was observed with the same frequency in all groups, which was different from the situation in healthy people. We did not find out a significant relationship between *MTHFR* genotype and the rate of thrombotic complications. For MPD patients with normal and elevated HC concentration in blood serum it was shown, that factor VIII level was higher in HHC, than in patients with normal HC level (222±26,5% and 116±20%, p=0,002). The same was found for von Willebrand factor (202±15,6, 120±14,6%, p<0,003). Comparing the data on ADP induced platelet aggregation in patients with thromboses, it was shown that the activation of the later was higher in HHC against normal data of blood HC (77 ±15,7% and 47±12,1%, accordingly, p<0,001). Regressional analyses showed that only HHC has a statistically significant influence on thrombotic complications rate for MPD patients (p=0,004). We consider that the lowering of HC plasma levels together with vitamin therapy were the more expressed, the higher was its baseline level. *Conclusion*. The relationship between HHC levels and thromboses in the patients with MPD was shown. We suppose that different stages of proliferative diseases may influence the HC levels in patients with MPD. Homocystein is a highly significant, independent risk factor for thrombotic complications. Therefore it is necessary to discover and treat HHC.

1081

ENDOGENOUS ERYTHROID COLONIES AND SERUM ERYTHROPOIETIN IN PATIENTS WITH CHRONIC MYELOPROLIFERATIVE DISEASES BEFORE AND DURING TREATMENT

T. Manakova,[1] N. Tsvetaeva,[2] M. Sokolova,[2] A. Levina,[2] N. Khoroshko[2]

[1]*Russian Academy of Medical Sciences, Moscow, Russian Federation;* [2]*Hematology Research Center, Moscow, Russian Federation*

Background. The chronic myeloproliferative diseases, polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF) share more similarities with each other and often difficult to distinguish from other causes of elevated blood cell counts. These diseases were shown to be clonal, arising from multipotent hemopoietic stem cell or progenitor and lacking of specific biological markers. Despite the clinical and pathologic features, the diagnosis of an individual patient with isolated erythrocytosis or thrompocytosis is often difficult. *Aims*. The aim of this study was to compare the serum erythropoietin (EPO) level and the growth of endogenous erythroid colonies (eBFU-E) in semisolid cultures of bone marrow and peripheral blood cells in patients with PV, ET and IMF. *Methods*. EPO level was determined in serum of 22 PV patients, 22 ET patients and 8 IMF patients at diagnosis and 16 PV, 19 ET and 8 IMF during/after the treatment. The growth of eBFU-E was evaluated *in vitro* in bone marrow and peripheral blood in 41 PV patients, 38 ET patients and 10 IMF patients at diagnosis and during/after treatment. 3 PV patients, 1 ET patient treated by phlebotomy only, and the others received myelosuppressive treatment (hydroxyurea or α-interferon) during 4 - 53 months. *Results*. In PV 77% patients at diagnosis and 62% patients receiving myelosuppressive therapy EPO level was below the normal limits. 85% untreated ET patients and 60% treated with myelosuppressive agents had subnormal EPO level. The low EPO values were detected in 87% untreated IMF patients and in 57% patients during/after treatment. Nineteen (95%) of 20 PV patients presented eBFU-E growth in bone marrow, and in 13 (72%) of 18 patients showed circulating eBFU-E at diagnosis but only 2 (16%) of 12 PV patients had bone marrow eBFU-E and in 13 (31%) of 42 patients circulating eBFU-E were observed after the treatment. In untreated ET patients 3 (33%) of 9 showed bone marrow eBFU-E and 3 (27%) showed eBFU-E in peripheral blood; in 5 (20%) of 24 ET patients in bone marrow and in 11 (29%) of 38 patients in peripheral blood eBFU-E were detected after the treatment. Among IMF patients, 3 (75%) of 4 were positive for bone marrow eBFU-E and all patients had eBFU-E in peripheral blood at diagnosis; 4 (66%) of 6 patients IMF and 4 (40%) of 10 patients had eBFU-E in bone marrow and peripheral blood, accordantly. Thus the low serum EPO level and the growth of eBFU-E in cultures of bone marrow and peripheral blood were maintaining in the most of PV, ET and IMF patients during and after treatment. The correlation was observed between EPO and endogenous growth of BFU-E in 5 (71%) of 7 PV patients in bone marrow and in 9 (47%) of 19 patients in peripheral blood. In ET patients the correlation was observed in 6 (33%) of 18 patients in bone marrow and in 11 (47%) of 23 patients in peripheral blood. The correlation between EPO and eBFU-E in IMF was found in 50% patients both in bone marrow and in peripheral blood. Conclusions. We conclude that EPO level and the growth of eBFU-E *in vitro* are useful in the diagnosis and monitoring of minimal residual disease of polycythemic conditions and diseases with increased platelet levels. The investigation of anomalous erythroid proliferation and regulation in patients with chronic myeloproliferative diseases were useful and reliable tools for the diagnosis and pathogenesis of these diseases.

1082

JAK2 V617F MUTATION AND CLINICAL BEHAVIOR IN PATIENTS WITH ESSENTIAL THROMBOCYTEMIA: RESULTS FROM A SINGLE CENTER STUDY

E. Balleari,[1] C. Passalia,[1] S. Pozzi,[2] S. Panarello,[1] P. Pitto,[1] F. Raggi,[1] F. Bertolotti,[2] P. Vacca,[1] M. Podest,[2] R. Ghio,[1] F. Frassoni[2]

[1]*Università di Genova, Genoa, Italy;* [2]*Centro Cellule Staminali e Terapia Cell, Genoa, Italy*

It has been reported that an acquired *V617F* mutation in *JAK2* occurs in approximately 30-50% of patients with essential thrombocytemia (ET). Recent reports suggest that the presence of this mutation is associated with clinical features resembling polycythemia vera as well as an increased risk of thrombosis. *Aim*: the aim of present study was to verify whether patients suffering from ET and positive for *JAK2 V617F* mutation (mutation-positive) display a distinct clinical behavior from those without *JAK2 V617F* mutation (mutation-negative). *Methods.* genomic DNA from peripheral blood granulocytes of patients with ET attending the outpatient Clinic were tested for the presence of JAK2-V617F mutation by an allele-specific Polymerase Chain Reaction (*Baxter EJ et al. Lancet 2005;365:1054-61*); their clinical records were reviewed for their diagnostic blood counts, thrombotic histories and bleeding events. Thrombotic complications included major thromboses as well as microvascular disturbances. *Results.* among the 33 ET patients in who was possible to assess the status of *JAK2 V617F* mutation, 23 (69.7%) were mutation-positive and 10 (30.3%) were mutation-negative. Blood counts at diagnosis and relevant clinical events are summarized in Table 1.

Table 1. Patient clinical characteristics.

	All patients	*Mutation positive*	*Mutation negative*	*p-value*
Number (%)	33	23 (69.7)	10 (30.3)	
Female/males	21/12	16/7	5/5	
Age at diagnosis (years)	60	64	48	p=0.01
Disease duration (months)	68	67	71	NS
Hemoglobin (g/dL) (±SD)	14.1 (±2.1)	14.7 (±1.2)	13.6 (±1.4)	p=0.03
WBC ($\times 10^9$/L) (±SD)	12.4 (±7.7)	13.4 (±8.7)	9.9 (±3.8)	NS
Platelets ($\times 10^9$/L) (±SD)	839 (±250)	903 (±180)	758 (±231)	NS
Subjects with thrombosis (%)	14 (42%)	9 (39%)	5 (50%)	NS
Subjects with bleeding (%)	7 (21%)	5 (22%)	2 (20%)	NS
Subjects with microvascular events (%)	8 (24%)	7 (30%)	1 (10%)	NS
Patients receiving cytoreduction (%)	28 (85%)	22 (96%)	6 (60%)	p=0.02

Mutation-positive patients were significantly older than mutation-negative; in mutation-positive patients blood counts at diagnosis were higher than in mutation-negative individuals, with a statistically significant difference for hemoglobin concentration. With a similar median follow-up of 67 and 71 months - respectively for mutation-positive and mutation-negative patients, no difference was observed in the occurrence of thrombotic or bleedings events between the two groups of patients, while a significantly higher number of mutation-positive patients required some form of cytoreduction at any time from the diagnosis. *Conclusions.* our study suggests that the presence of the JAK2 V617F mutation, particularly frequent in our series of ET patients, may identify a subgroup of patients with a more pronounced polycythemic behavior, as already suggested.

1083

CLINICAL COURSE OF 65 PATIENTS WITH MYELOFIBROSIS WITH MYELOID METAPLASIA: EXPERIENCE OF MONZA CENTER

E. Elli, M. Parma, A. Soccodato, E. Lanzi, E. Pogliani

Ospedale San Gerardo, Monza, Italy

Backgrounds. Myelofibrosis with myeloid metaplasia (MMM) is a chronic myeloproliferative disorder, characterized by bone marrow reactive fibrosis, extramedullary hemopoiesis, progressive anemia and marked splenomegaly. Overall median survival ranges from 3,5 to 5,5 years, according to the presence or absence of adverse prognostic factors, with final evolution toward disease progression (DP) or leukemic transformation (LT). *Aim and Methods.* we analysed 65 MMM patients (pts) referred in our hematology unit from 1999 to 2005, in order to provide information about initial features, treatment, clinical course and survival. *Results.* The median age at diagnosis was 66 years (range 39-79) with 17 pts (26%) aged less than 55 years and a M/F ratio of 44/21. 39 pts (60%) presented idiopathic MMM, 26 (40%) MMM secondary to Polycythemia Vera (7) or Essential Thrombocythemia (19). At diagnosis, spleen enlargement (median 5 cm, range 1-30) below costal margin was present in 52 pts (80%). The median value of WBC was $11,9\times10^9$/L, of Hb was 11,6 g/dl, of platelets was 358×10^9/L. 48 pts (74%) had circulating myeloid precursors; 21 (32%) pts had blasts. The median value of LDH was 931 U/L and the median count of $CD34^+$ cell was $59,8\times10^6$/L (evaluated on 37 pts). According to disease status, 15 pts (23%) received no treatment, 13 (20%) supportive care alone, 9 (14%) androgens or steroids, 23 (35%) anti-platelet drugs and 27 (41,5%) myelosuppressive agents alone or in combination with the above treatment. Eight pts (12%) underwent splenectomy after a median of 11,5 months from diagnosis. Only 3 pts underwent allogeneic stem cell transplantation. 50 pts (77%) are actually alive after a median follow-up of 28 months (range 3-84). According to Dupriez scoring system, 4 pts (6%) were assigned to high risk (HR), 25 (38,5%) to intermediate risk (IR) and 36 (55,5%) to low risk (LR) group. The median survival was 14, 24 and 34 months for HR, IR and LR group, respectively. The median survival in pts aged less than 55 years was longer (38 months) with significant difference between LR (59 months), IR (28 months) and HR (15 months) pts. Fifteen pts (23%, 3 pts aged less than 55 years) died, 6 for LT or DP, 4 for thrombosis or bleeding, 3 for secondary cancer and 2 for heart failure. At time of LT and DP, most pts worsened organomegaly and constitutional symptoms and presented higher LDH levels than at diagnosis. *Conclusions.* our experience confirms different outcome of MMM pts according to Dupriez score. Younger patients have a longer median survival. DP and LT correlate with increase of spleen volume, onset of constitutional symptoms and higher LDH levels respect to diagnosis.

1084

THE CD44 MAB A3D8 INDUCES APOPTOSIS AND G1 CELL CYCLE ARREST IN NEOPLASTIC MAST CELLS IN SYSTEMIC MASTOCYTOSIS

A. Boehm, S. Florian, K. Sonneck, A. Gruze, W.F. Pickl, P. Valent, W.R. Sperr

Medical University of Wien, Wien, Austria

Backgrounds. Recent data suggest that CD44 may serve as a new therapeutic target in AML and possibly also in other myeloid neoplasms. Systemic mastocytosis (SM) is a myeloid neoplasm characterized by abnormal growth and accumulation of mast cells (MC) in one or multiple organs. We have previously shown that normal tissue MC express CD44. *Aims.* In the present study, we asked whether CD44 is expressed on neoplastic human MC and whether CD44-ligation by the monoclonal antibody (mAb) A3D8 would be associated with inhibition of growth of neoplastic MC. Methods and Results. As assessed by flow cytometry, primary neoplastic MC were found to express CD44 in all patients with SM analyzed (n=10). The human mast cell leukemia (MCL) cell line HMC-1 was also found to express CD44. As assessed by ^{3}H-thymidine incorporation, the CD44 mAb A3D8 decreased the proliferation of HMC-1 cells in a dose-dependent manner (A3D8, 5 μg/mL: 46±26% of control=100%, $p<0.05$). Similar effects of A3D8 were observed with primary neoplastic MC obtained from a patient with MCL (A3D8, 5 μg/mL: 68±20%) and one with smouldering SM (A3D8, 2.5 μg/mL: 42±7% of control, $p<0.05$). To analyze the mechanism of A3D8-induced growth inhibition, cell survival and cell cycle distribution were analyzed. In these experiments, CD44-ligation induced an approximately 3-fold increase in apoptotic HMC-1 cells compared to control. As assessed by flow cytometry, we were also able to demonstrate that A3D8 induces cell cycle arrest in the G1-phase. *Conclusions.* In summary, our results suggest that CD44-ligation is followed by inhibition of growth of neoplastic human MC through induction of apoptosis and G1 cell cycle arrest. Whether targeting of CD44 in neoplastic MC in patients with high grade MC disorders is of clinical significance remains to be determined in forthcoming studies.

1085

SYSTEMIC MASTOCYTOSIS WITH EOSINOPHILIA (SM-EO): CLINICAL SIGNIFICANCE OF MOLECULAR MARKERS AND ORGANOPATHY

A. Boehm, M. Foedinger, F. Wimazal, M. Mayerhofer, W.R. Sperr, H. Esterbauer, P. Valent

Medical University of Wien, Wien, Austria

Backgrounds. In a group of patients with systemic mastocytosis (SM), marked and sustained eosinophilia is detectable (SM-eo). Although the molecular defect has been defined for some of these patients, little is known about the impact and clinical correlates of eosinophilia in SM. Methods. In a cohort of 61 patients with SM, we identified 11 with permanent eosinophilia (>1,500/μL). According to the WHO-classification, 4 had indolent SM (ISM), 1 smouldering SM (SSM), 2 SM with associated chronic eosinophilic leukemia (SM-CEL), and 4 aggressive SM (ASM). Results. In the 2 patients with SM-CEL, the FIP1L1/PDGFRα fusion gene-product was detectable, but no KIT mutation at codon 816 was found, whereas in most other SM-eo patients, KIT D816V, but not *FIP1L1/PDGFRα*, could be detected. Other molecular defects including *BCR/ABL*, *CBFβ/MYH11*, *JAK2 V617F*, or a monoclonal T cell receptor rearrangement were not detected in patients with SM-eo. In the two patients with SM-CEL, fatal organopathy of the heart developed. By contrast, in all other SM-eo patients, organopathy, if recorded, affected the bone marrow, liver, or skeletal system, but did not affect the heart, even if eosinophilia persisted for many years. Conclusions. Our data show that the biochemical basis of eosinophilia in SM is variable and correlates with organopathy. SM-eo thus is a prediagnostic checkpoint but not a final diagnosis. For correct final diagnosis and selection of targeted drugs, it is important to apply molecular markers including FIP1L1/PDGFRα in SM-eo.

1086

DIAGNOSTIC AND CLINICAL RELEVANCE OF CHRONIC MYELOPROLIFERATIVE DISORDERS PERIPHERAL CELLS ANTIGENS

C. Buquicchio, M. Santoro, T. Patruno, A. Iacobazzi, G. Tarantini, D. Santorsola, A. Riezzo

Hematology, Trani, Italy

Backgrounds. Immunophenotype of peripheral blood cells in patients with chronic myeloproliferative disease (CMD) has not been extensively investigated to recognise association between CMD subtype , phase of disease and peripheral cells antigens. *Aim*. The aim of our study is the identification of same cytofluorimetry parameters useful to follow up patients with CMD. *Methods.* We analized the immunophenotype of 20 consecutive patients observed in our institute, during cytoreductive treatment for CMD. Of 20 patients 9 had essential thrombocythemia (ET), 7 myelofibrosis (MF) and 4 atypical myeloproliferative disorders (AMD). Flow cytometry was performed on pheripheral blood sample using double or triple platform assay to identify CD34, CD33, CD61, CD42a positive and CD33/34 coexpression on CMD patients cells. *Results.* The main hematologic characteristics analized in CMD patient about white blood cells and platelet count range were in ET group WBC $6.9\text{-}16.1\times10^9$/L;Plt $487\text{-}1976\times10^9$/L, in AMD group WBC $4.26\text{-}9.8\times10^9$/L;Plt $171\text{-}337\times10^9$/L, in MF group WBC $8.55\text{-}26.47\times10^9$/L;Plt $9\text{-}1595\times10^9$/L, respectively. The median of circulating CD34, CD33, CD33/34,CD61, CD42a, expressed in 106 cells were in ET group (20, 117, 0, 463, 469 respectively), in AMD group (54, 79, 4, 553, 435 respectively) , in MF group (63, 261, 33, 145, 371 respectively). We observed higher expression of CD33 than CD34 in CMD patients studied and a significantly elevated median number of circulating CD33 and CD34 in patients with MF, especially not responsive to cytoreductive treatment and inverse relationship between absolute number of CD33 positive and platelet count. Instead we evaluated a statistical correlation between of CD 61 and 42a positive cells and thrombocytosis in ET and AMD groups than others CMD patients. *Summary/Conclusions.* These initial data reflect the increased number of circulating CD33 and CD 34 positive cells in patients with MF not responsive to treatment. This consideration may show an abnormal function of bone marrow for a hematopoietic differentiation decline. Further studies with a larger number of patients could improve the identification of surface antigens to classify better subclass of CMD associated to clinical features and to predict prognostic evolution of disease.

1087

ASSESSING THE BENEFIT/RISK BALANCE OF LONG-TERM ANAGRELIDE HYDROCHLORIDE (XAGRID/AGRYLIN) TREATMENT FOR ESSENTIAL THROMBOCYTHAEMIA

R. Petitt

Mayo Clinic, Rochester, USA

Backgrounds. Essential thrombocythaemia (ET) is a myeloproliferative disorder (MPD) associated with thrombohaemorrhagic complications. The disease uncommonly progresses to myelofibrosis or acute leukaemia; the risk of transformation may be increased by cytoreductive treatments. Anagrelide (Xagrid™/AgrylinTM) is a non-cytotoxic agent that selectively reduces platelet production. Its long-term efficacy and safety in patients with MPD (maximum duration of exposure, 7.1 years) have been previously described.[1] *Aims.* To assess the benefit/risk balance of long-term anagrelide therapy in patients with ET. *Methods.* Retrospective analysis of an open-label, multicenter anagrelide trial. *Results.* Data from 3660 patients with MPD (2251 with ET) were included in the safety analysis. The maximum duration of followup was 11.4 years. Prior myelosuppressive therapy had been administered in 81% of patients (reasons for change to anagrelide, toxicity [33%] and poor platelet control [31%]). Efficacy data were available for 934 patients with ET. A response rate of 78.7% was observed (67.2% complete response [decrease in platelet count to ≤600×10^9/L or a decrease of ≥50% from baseline within 4 weeks of the start of anagrelide therapy]; 11.5% partial response [decrease of 20% to <50% from the baseline value at least 4 weeks after starting anagrelide]).[2] Response rates for patients who had failed previous therapy and for patients who were intolerant of previous therapy were similar (78.8% and 75.6%, respectively). After the first year, platelet count decreases were well maintained. Results were similar for both sexes, different age groupings, and ethnic origins. At baseline, 163/934 (17.5%) patients reported ET-related symptoms, including GI and other bleedings, arterial or venous thromboses, angina, pulmonary embolism, transient ischaemic attacks, peripheral ischaemia, and paraesthesia).3This had reduced to 7.9% (63/796, p<0.001) after 12 weeks and was maintained during follow up (3.2% [15/470] at 1 year and 2.5% [6/239] at 2 years, p<0.001). Adverse events occurred in 40.2% of the patients and were generally mild. Anagrelide was discontinued in 38.6% of patients (adverse events accounting for 29.2% of patients stopping treatment). Transformation to AML/MDS occurred in 47/2251 (2.1%), but only in subjects who had perviously been exposed to cytoreductive treatment. The observed mortality rate (8.8%) was consistent with that which would be expected in ET patients. The most common reasons for death (≥1%) were CML, reason unknown or unspecified, and sepsis. *Summary/Conclusions.* Anagrelide effectively reduces platelet counts and thrombohaemorrhagic complications in patients with ET. This is independent of gender, age, ethnic origin, and prior therapy. The drug demonstrates a recognized safety profile. Benefits are maintained during long-term follow-up without an increase in disease transformation.

References

1. Fruchtman SM et al. Leuk Res 2005;29:481-91.
2. Fruchtman SM et al. Presented at the 45th Annual Meeting of the American Society of Hematology. December 6-9, 2003, San Diego, California, USA.
3. Petitt RM. Presented at the 45th Annual Meeting of the American Society of Hematology. December 6-9, 2003, San Diego, California, USA.

1088

TREATMENT WITH HYDROXYUREA AS SINGLE AGENT DOES NOT INCREASE THE RISK OF SECOND MALIGNANCIES IN ESSENTIAL THROMBOCYTHEMIA PATIENTS

F. Radaelli,[1] F. Onida,[2] F. Rossi,[2] M. Colombi,[2] V.R. Zilioli,[2] F. Somalvico,[2] A. Zanella[2]

[1]*Ospedale Maggiore Policlinico, Mangiagalli, Milano, Italy;* [2]*Ospedale Maggiore Policlinico, Milano, Italy*

Among chronic myeloproliferative disorders, Essential Thrombocythemia (ET) has the most favourable prognosis, with thrombotic events and second malignancies representing major causes of death. Several studies have underlined the association between treatments with chemotherapeutic agents, especially alkylating drugs, and the risk of developing second malignancies in patients with ET. To retrospectively investigate long-term development of second malignancies in ET patients, analysing possible associations with cytoreductive treatments Data from 331 patients with diagnosis of ET (according to the PVSG and WHO criteria) referred to our institution from January 1977 to November 2002 were retrospectively analysed. Patients were grouped according to the type of treatment received. Estimates of survival were based on the Kaplan-Meier method. Cumulative doses of HU, Melphalan and Busulphan were categorized as *low* or *high* based on median values for each drug. A logistic regression model was then applied including occurrence of malignancy during follow-up as the dependent variable and HU, Melphalan/Busulphan (ALK) and use of HU after ALK, as the independent variables. The population included 214 females and 117 males, with a median age of 61 years and a median follow-up of 108 months. Median survival of the whole population was not reached, with more than 60% of patients still alive 240 months after diagnosis. After referral, 137 patients did not receive any treatment, whereas 194 were treated with chemotherapy; 116 patients received only HU, 38 only ALK, and 40 ALK followed by HU. In the whole population, second malignancies (11 acute leukemias, 4 NHL, 28 solid tumors) were detected in 43 patients (13%), with a median time from diagnosis of 87 months (range 23 to 184). In particular, we observed 10 cases (7.3%) among untreated patients, 13 cases (11.2%) among patients treated only with HU, 10 cases (26.3%) among patients who received only ALK, and 10 cases (25%) among patients treated with ALK+HU. Therefore, the proportion of second malignancies was higher (p<0.01) in patients who received ALK as part of their treatment, whereas no significant difference was documented between patients who did not receive any treatment and patients who received HU as single agent. By multivariate analysis, high dose of Melphalan was statistically different with respect to no use of Melphalan (Wald test=12.1 p value=0.0005, odds ratio 6.56 with 95% confidence interval from 2.27 to 18.94); high dose of Busulphan was statistically different with respect to no use of Busulphan (Wald test=4.4 p value=0.0370, odds ratio 3.67 with 95% confidence interval from 1.08 to 12.47). HU was not significant with both doses. Use of HU after ALK was not significant. Similar findings were observed when statistics were applied only to patients with haematological malignancies. This study, derived from a large population of ET patients with long-lasting observation times, shows that treatment with single agent HU does not increase the risk of second malignancies, in particular of haematological subtype. On the contrary, our results confirm the notion that cumulative high dose of ALK associate with high risk of developing second cancers.

1089

THE COMBINATION OF FLUDARABINE, ARA-C, IDARUBICIN AND GEMTUZUMAB-OZOGAMICIN (MY-FLAI) IS A SAFE AND EFFECTIVE THERAPY FOR ELDERLY AML PATIENTS.

A. Albarello, S. Aquino, G. Catania, R. Varaldo, F. Olcese, M. Clavio, I. Pierri, M. Miglino, S. Biasco, M. Risso, G.L. Michelis, M. Balocco, P. Canepa, A.M. Carella, M. Sessarego, M. Gobbi

Department of Hematology-Oncology, Genoa, Italy

Background. Elderly AML patients and patients with AML evolved from MDS or therapy related display a very poor prognosis. In the last decade the association of fludarabine, Ara-C and anthracycline proved to be an effective and well tolerated induction regimen for this group of patients and, more recently, the introduction of gemtuzumab ozogamicin has opened new perspectives in the treatment of AML. *Methods*. We report here our preliminary experience on 18 elderly AML patients treated as first line therapy with MY-FLAI regimen (Fludarabine 25 mg/m^2, Ara-C 1 g/m^2, idarubicin 5 mg/m^2 all for 3 days, followed by gemtuzumab ozogamicin 3 mg/m^2 at day 4). Responding patients received the same regimen as consolidation therapy. *Patients*. The median age of patients was 66 (range 54-76); M/F ratio was 8/10; FAB subtypes were M0 in 1 patient, M1 in 8, M2 in 5, M4 in 2, M5 in 1, M6 in 1. Nine patients had de-novo AML (50%); in nine patients AML was secondary to NHL (3), MDS (4), epithelial neoplasms (2). Hematological parameters before therapy were the following: WBC 6×10^9/L (range 1,7-110); Hb 9,4 g/dL (7,8-12); Plt 40×10^9/L (15-190). Cytogenetic analysis revealed a poor prognosis alteration in 9 patients (50%, with 7 complexes karyotypes) and an intermediate alteration in the other 9 patients. Results. The neutrophil (PMN > 0,5×10^9/L) and platelet (> 25×10^9/L) recovery required a median of 18 (range 11-23) and 18 days (range 10-29) from the end of therapy. The median number of days with fever (> 38°C) was 5. Therapy has been well tolerated. Neither deaths during induction occurred nor severe infectious, hepatic or cardiac complications were recorded. The median hospitalization period was 32 days (range 19-56). Eleven pts (60%) achieved CR, 7 (40%) were refractory. Complete remission and

survival had a median lenght of 6 months (range 2-19) and 8 months (range 3-20), respectively. Five out of 11 patients have relapsed and 9 have died of disease. Poor prognosis cytogenetics had a negative impact on CR rate (44% compared to 77% in patients with intermediate prognosis karyotype), whereas de-novo and secondary AML had similar CR rates (55% and 66%, respectively). *Conclusions*. Considering the reported outcome of elderly AML patients treated with conventional chemotherapy and the unfavourable clinical features of this series (high percentage of unfavorable karyotype and secondary AML) our preliminary results show that MY-FLAI may represent a well tolerated and effective induction and consolidation regimen for elderly non M3 AML patients.

1090

EFFICACY AND SAFETY OF BORTEZOMIB IN PATIENTS WITH REFRACTORY AND RELAPSED MULTIPLE MYELOMA OUTSIDE CLINICAL TRIALS: RESULTS FROM THE CATALAN MYELOMA/AMYLOID STUDY GROUP (GEMMAC) IN 120 PATIENTS

M.T. Cibeira,[1] A. Garcia,[2] L. Rosinol,[2] J. Petit,[2] A. Oriol,[2] E. Abella,[2] J.A. Soler,[2] E. Plensa,[2] M. Callis,[2] Y. Gonzalez,[2] J. Macia,[2] J. Orriols,[2] L. Escoda,[2] G. Heras,[2] A. Sureda,[2] J. Bladé[2]

[1]*Hospital Clnic, IDIBAPS, Barcelona, Spain;* [2]*The Catalan Myeloma/Amyloid Study Group, GEMMAC, Spain*

Background. Bortezomib (Velcade) has recently been approved for the treatment of refractory and relapsed multiple myeloma (MM). In this setting, a response rate ranging from 35 to 50% has been reported in patients included in prospective clinical trials. However, the data on the efficacy and safety of bortezomib outside the context of clinical trials are limited. *Aim*. To analyze the efficacy and safety of bortezomib therapy in refractory or relapsed MM patients treated in community practice. *Patients and Methods*. Between August 2003 and February 2006, 120 patients (63M/57F, median age: 63 years) with refractory or relapsed MM were treated with bortezomib outside the context of clinical trials in 16 centres in the area of Catalonia. Fifty-five (46%) patients had untreated relapse, 38 (32%) refractory relapse and 27 (22%) primary refractory disease. Twenty-seven of them (22%) had extramedullary plasmacytomas. The median number of previous lines of therapy was 2 (range: 1-6). Fifty patients (42%) had received high dose therapy followed by stem cell transplantation (HDT/SCT): single autologous (35), double autologous (6), autologous followed by allogeneic with reduced-intensity conditioning (6) and allogeneic (3). Bortezomib was administered intravenously at a dose of 1.3 mg/m2 on days 1, 4, 8, and 11 of every 21-day cycle. Six patients who had no response after two cycles of bortezomib alone continued treatment receiving also oral dexamethasone. The median number of cycles administered was 3.5 (range: 1-13). At the time of this analysis, bortezomib therapy was still ongoing in 22 cases, and 12 patients were not yet evaluable for response. Responses were evaluated according to the EBMT criteria. *Results*. Among the 108 already evaluable patients, the response rate to bortezomib was 52% (57/108), with 7 (6%) complete, 38 (35%) partial and 12 (11%) minimal responses. The remaining 51 patients showed no response: no change (18), progressive disease (20), and early death within the first two months from bortezomib onset (13). The median time to best response was 3.5 months (range: 0.5-11). Grade 3 or 4 adverse events, which occurred in 45% of evaluable patients, included: thrombocytopenia (30%), asthenia (12%), peripheral neuropathy (8%), gastrointestinal symptoms (4%), fever (4%), postural hypotension (2%), rhabdomyolysis (1%) and tumour lysis syndrome (1%). The drug was discontinued because of side effects in 10 patients: peripheral neuropathy (7), asthenia (1), thrombocytopenia (1) and unknown (1). After a median follow-up of 7.4 months (range: 1.6-30.4), 15 of the 57 responding patients had relapsed (26%). *Conclusion*. In this observational study, the response rate to bortezomib in patients with relapsed and refractory MM treated in community hospitals was comparable to that achieved in the recently reported prospective clinical studies. Toxicity was manageable, but led to bortezomib discontinuation in 10% of the patients. In the present series, the evaluation of time to progression and overall survival requires longer follow-up.

1091

INHERITED COAGULATION DISORDERS IN CENTRAL PART OF IRAN

M. Mojtabavi Naini, F. Derakhshan, F. Makarian Rajabi, H. Hoorfar, R. Derakhshan

Isfahan University of Medical Sciences, Isfahan, Iran

Backgrounds. the incidence of hereditary coagulation disorders may vary according to the country and ethnic origin. Demographic datasets are vital in setting priorities, allocation of resources, measurement of outcomes, and comparison of alternate approaches. Aim: The aim of this study was to document the epidemiological features, disease severity and complications associated with inherited coagulation disorders in central part of Iran. Methods. A comprehensive survey was undertaken in January 2006. Clinical history, Laboratory and treatment data, and long term complications of all cases (553 persons) diagnosed with inherited coagulation disorders, were studied in Hematology-Oncology Department, Isfahan University of Medical Sciences. *Results.* 465 male and 88 female with Mean±SD age of 23.4±12.9 were studied. Hemophilia A was found in 341(61.7%), 48 (8.7%) had hemophilia B, 74 (13.4%) had Von Willebrand disease, and 34(6.1%) had platelet dysfunctions. The rare coagulation disorders (n=88) include 30 patients with FV deficiency, 23 with FVII, 13 with afibrinogenaemia, 10 with FX. Among them 19 (3.4%) had combined FVIII and FV deficiency. 228 (41.2%) patients had severe hemophilia. The most common complications were Epistaxis (n=59), Hemartrosis (n=51) and Hemophilic Arthropathy (n=49).None of the patients were human immunodeficiency virus positive but 125 (22.6%) were hepatitis C virus positive and 2 (0.4%) were hepatitis B positive. Replacement therapy primarily relied on Cryoprecipitate and Fresh Frozen Plasma. 27 patients showed factor inhibitor arising. *Conclusion.* Most of the hemophilic patients have the severe type of the disease, this differs from that obtained by other studies elsewhere and it may be due to some degree of under diagnosis of the less severe forms of hemophilia. Implement a program of prophylaxis for hemophilic arthropathy in children with severe hemophilia could be helpful. A more stringent policy for blood product usage, HCV screening and HBV vaccination is needed to abolish these diseases in patients with hemophilia.

1092

DISSEMINATED INTRAVASCULAR COAGULATION IN AN ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA

A. Triffet,[1] P. Cauchie,[1] A. Chaikh,[1] B. Woodhams,[2] M. Van Haeverbeek,[1] D. Brohée[1]

[1]*Centre Hospitalier Universitaire, Charleroi, Belgium;* [2]*Stago R&D, Paris, France*

Introduction. Disseminated intravascular coagulation (DIC) is a syndrome suggested by clinical signs and laboratory tests. The diagnosis may be based on the new ISTH overt-DIC score or other parameters including soluble fibrin monomer complexes (SFMC), antithrombin and protein C consumption. Angioimmunoblastic T-cell lymphoma (AITL) is an uncommon lymphoma. We report the case of a 71-year-old woman who presented AITL with an inaugural DIC associated with additional adverse prognosis parameters like SFMC, antithrombin III (AT) and protein C levels. Clinical observation. This woman was admitted to the hospital for severe health alteration. Clinical examination showed pallor, diffuse enlarged peripheral lymph nodes, hepato-splenomegaly, purpuric vasculitis and bruising. Laboratory analyses showed (→ nadir): hemoglobin 9→7g/dL (nr: 12-16), platelets 64→39×10^9/L (nr: 150-400), lactic dehydrogenase (LDH) 1272 UI/L (nr: 240-480). Coagulation tests were compatible with DIC (ISTH score 5 up to 8): serum fibrinogen was 1.48→0,55g/L (nr: 2-4,5). APTT time was 35→47, PT was 55→37% (nr: 70-120), AT was 42%_34% (nr: 80-120), D-dimers were up to 12,95→g/mL (normal <0,5). STA-liatest FM, a new immuno-turbidimetric method of fibrin monomer, was positive during the time of DIC, up to 17 µg/mL (normal <6) while F-test was positive once. Autoimmune features included severe not-HIV CD4 lymphopenia (80/mm^3), positive Coombs test and polyclonal gpathy (41g/L). Chest and abdominal CT-scans showed diffuse adenomegaly, hepatosplenomegaly and ascites. A lymph node biopsy showed diffuse infiltration by CD3+, CD4+, CD20, CD10- T-cells. Cytometry revealed T- and B- activation with 73% HLA-DR+ T, 15% CD25+ B, 11% CD38+++ B, and 5% of plasma cells. PCR was negative on a paraffin 'block. A diagnosis of angioimmunoblastic T-cell lymphoma Ann Arbor stage IV with DIC was made. An oral chemotherapy (methylprednisolone 1 mg/kg/day and cyclophosphamide 50mg/day) was given. Two weeks later, DIC resolved with negativity of F-test. Two months later, the patient was in complete clin-

ical remission with LDH normalisation. *Conclusion.* AITL is a rare disease. This report is the 3rd case described in the literature with a *de novo* DIC. DIC was followed by different criteria with a discordant evolution. Indeed, in this case, evolution of coagulation tests and DIC score was not parallel with an early decrease of STA-Liatest FM. More studies are warranted.

1093

DERANGEMENT OF HEMOSTATIC PROTEINS IN HCV CIRRHOTIC PATIENTS: RELEVANCE TO HEMORRHAGIC DIATHESIS AND THROMBOTIC EPISODES

E.I. El-Bassiouni, A.E. El Bassiouny, A. Taha, R. El-Khayat

Theodor Bilharz Research Institute, Giza, Egypt

Backgrounds. An altered coagulation profile resulting in decreased natural anticoagulant levels leading to haemostatic activation is described in patients with liver cirrhosis. The protein C system, a major physiologic regulator of haemostatic balance, controls thrombin production and guards against thrombotic episodes. *Aims.* This study was designed to assess the components of protein C system in HCV cirrhotic patients to determine whether alterations in these haemostatic proteins are related to degree of hepatic dysfunction and/or haemostatic activation and development of hemorrhagic diathesis or thrombotic episodes. Methods. Components of protein C (PC) system were assessed in 44 patients with hepatitis C virus liver cirrhosis, of whom 15 patients had acute hematemesis and 14 patients had portal vein thrombosis (PVT). According to Child-Pugh criteria, all patients were graded Child C. Neutrophil elastase (NE) release was determined by measuring elastase-α-1-proteinase inhibitor (E-α-1-PI) complex using an immune activation assay. Levels of tumor necrosis factor-α (TNF-α), PC antigen (PC Ag), total protein S (TPS), free protein S (FPS), soluble thrombomodulin (TM), tissue-plasminogen activator (t-PA), t-PA-PAI-1, plasmin-α-2-antiplasmin (PAP), thrombin-antihrombin III (TAT) and D-dimer (D-D) complexes were measured in plasma by ELISA. Fibrinogen level, functional activities of PC (PC Ft), plasminogen activator inhibitor-1 (PAI-1) and C4b-binding protein (C4b-BP) concentrations were also assessed. Results. Stimulation of the inflammatory process (increased TNF-α, NE and C4b-BP), endothelial injury (elevated TM and t-PA), reduction in anticoagulant proteins (low PC and PS), hypercoagulation and thrombin generation (elevated TAT and D-D), increased consumption (prolongation of coagulation screening tests, thrombocytopenia, hypofibrinogenaemia and decreased PC Ft/PC Ag ratio) and accelerated fibrinolysis (increased PAP, free t-PA and t-PA/PAI-1 ratio and decreased PAI-1) were detected in different cirrhotic groups compared to controls (15 healthy subjects). The haemostatic defects correlated with the marked elevation of inflammatory mediators and were more pronounced ($p<0.05$) in patients with PVT. A significant decline ($p<0.05$) in fibrinogen concentration and PC Ft/PC Ag ratio associated with a significant increase ($p<0.05$) in TAT and D-D levels was detected in bleeders with acute hematemesis and patients with PVT compared to cirrhotics with haemostatic balance. Moreover, FPS and PAI-1 levels were significantly elevated ($p<0.05$) in patients with PVT compared to those with acute hematemesis and were inversely correlated with platelet count ($p<0.01$). *Conclusions.* These findings suggest that NE and TNF-α contribute to haemostatic alterations in patients with viral hepatitis C liver cirrhosis, and emphasize the clinical significance of protein C as a sensitive parameter for hepatic dysfunction and protein S and PAI-1 as reliable prethrombotic markers in these patients.

1094

SCREENING TEST FOR VON WILLEBRAND DISEASE IN CHILDREN: A PFA-100 CLOSURE TIME

M. Benedik-Dolnicar, A. Trampu-Bakija, V. Velenek-Prestor

Children's hospital Ljubljana, Ljubljana, Slovenia

Von Willebrand disease (VWD) is the most common inherited bleeding disorder so clinical symptoms, positive family history and good sensitive laboratory assays should be used for diagnosis. Definitive diagnosis of type 1 von VWD remains a problem. There is a temporal variability in the level of von Willebrand factor (VWF) in association with stress, inflammation, drugs and pregnancy. Manny patients with mild type 1 VWD sometimes have laboratory results in normal range and up to 40% of patients remain undiagnosed because of mild symptoms and borderline laboratory values. We evaluated the sensitivity of closure time (CT) of the PFA-100® system with both cartridges (collagen/epinephrine (EPI) and collagen/ADP (ADP)). Over a 5 year period (2000 - 2006) testing was performed on blood samples from 44 patients (age 0,3 - 19 years, median 12 years; 18 males and 26 females) registered in the Center for haemophilia and other bleeding disorders at the Children's hospital in Medical centre Ljubljana, Slovenia. In house reference ranges for children population were previously established and are 78 - 160s for EPI test and 55 - 124s for ADP CT. We found that all 6 patients with type 2 or 3 VWD had prolonged CTs with both EPI and ADP cartridges. Among 38 patients with definite type 1 VWD 31 patients had prolonged CT with either of cartridges. The sensitivity of test for type 1 VWD was 82%. 29 of them (76%) had prolonged CT with EPI cartridge and 23 (59%) with ADP cartridge. On the day of CT testing 7 of 38 patients (18%) with type 1 VWD had results in normal range with both cartridges. Only 3 of these patients had low VWF level, other 4 (10%) had normal VWF level. Sensitivity of the PFA-100® system established in our patients with type 1, 2 and 3 VWD was 84%. When clinical suspicion is strong, testing for CT and VWF level should be repeated in spite of normal CTs and normal VWF level.

1095

ANGIOGENIC FACTORS PATTERN IN LYMPHOCYTIC LEUKEMIA

S. Aref,[1] O. Salama,[1] Y. Al-Tonbary,[2] H. Mourkos[2]

[1]*Mansoura University, Mansoura, Egypt;* [2]*Mansoura Faculty of Medicine, Mansoura, Egypt*

Backgrounds. Angiogenesis is a crucial event in development and progression of solid tumors. Although the role of angiogenesis and angiogenic status is well studied in acute myeloid leukemia, its role in lymphocytic leukemia remains insufficiently characterized. *Aim.* is to investigate the profile of the systemic components of angiogenic factors in adult acute lymphoblastic leukemia at diagnosis (n= 25), in remission (n= 14), and chronic lymphocytic leukemia at diagnosis (n= 15), in remission (n= 9) in order to determine their clinical validity. *Methods.* By ELISA technique, we assessed the serum vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), endostatin and basic fibroblast growth factor (bFGF) levels in culture supernatants of peripheral blood mononuclear cells collected from ALL and CLL patients at diagnosis and in remission. On the other hand serum matrix metalloproteinase-9 (MMP-9) was assayed in remission only. *Results.* In ALL patients, sVEGF were significantly lower than control ($p<0.001$) and increased near control levels in remission ($p>0.05$). In contrast, bFGF level was significantly higher than that in control ($p=0.05$) and decreased near control level in remission ($p> 0.05$). Both serum TNF-α and endostatin levels showed no significant difference both at diagnosis (P>0.05) and in remission ($p >0.05$) comparing to control level. Serum MMP-9 level was significantly lower than that in control ($p=0.004$). In CLL patients, serum *VEGF, TNF-α* and *bFGF* levels in culture supernatant were significantly higher than control ($p<00.1$; $p= 0.007$; $p= 0.002$ respectively) and decreased near control level in remission ($p>0.05$ for All). Serum endostatin levels showed no significant difference at diagnosis and in remission comparing to control level ($p>0.05$). Serum MMP-9 level at diagnosis was significantly higher than that in controls ($p=0.009$). In conclusion: The biology of Angiogenic factors in adult ALL appears different from CLL. Although the angiogenesis have a vital role in CLL its role in adult ALL is not so clear.

1096

ASSOCIATION OF PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1) GENE POLYMORPHISM AND CHANGES IN PAI-1 PLASMA CONCENTRATIONS WITH STROKE

W.Y. Almawi,[1] S. Saidi,[2] L.B. Slamia,[3] S. Ben Ammou,[3] I.K. Al-Absi,[1] T. Mahjoub[2]

[1]*Arabian Gulf University, Manama, Bahrain;* [2]*University of Monastir, Monastir, Tunisia;* [3]*Hopital Farhat Hached, Sousse, Tunisia*

Background. Stroke is a major cause of morbidity and mortality, and rates as one of the leading causes of death and disability. As inhibitor of fibrinolysis, high levels of plasminogen activator inhibitor type 1 (*PAI-1*) reportedly increased the risk of cardiovascular disease, including stroke. Several factor influence *PAI-1* levels, including the 4G/5G polymorphism of which the 4G allele is associated high plasma levels of PAI-1, and discordant results were reported on the association of the PAI-1 4G/5G polymorphism with stroke. *Aims.* Insofar as aberrant fibrinolysis was reportedly associated with heightened stroke risk, the aim of the study was to determine the allele, genotype, and haplotype distribution of the 4G/5G and G[-844]A *PAI-1* polymorphisms in stroke patients, and to assess the contribution of these genotype on PAI-1 and t-PA antigen levels. *Methods.* This was a case-control study performed on 173 patients aged 32-84 years with first ischemic stroke, confirmed

by CT. Exclusion criteria included non-atherosclerotic causes, and patients on oral anticoagulants. Controls (n=271) were age -and sex-matched, without a history of stroke. Genotyping was done by PCR-SSP (4G/5G) or PCR-RFLP using Xho I (G[-844]A); PAI-1 and t-PA levels were assayed by ELISA. *Results*. Higher frequencies of the 5G allele (p=0.024; RR=1.72) and the -844 A/A genotype (p=0.032; OR=1.71; 95% CI=1.07-2.73) was seen in patients, while higher frequencies of the -844G allele (p=0.023; RR=0.584) and the 4G/4G genotype (p=0.03; OR=0.58; 95% CI=0.36-0.94) were found among control subjects. Complete linkage disequilibrium was seen between the 4G and -844G alleles, and between the 5G and -844A alleles in patients (p=0.022). While *PAI-1* antigen levels were increased in 4G/4G, more than -844 A/A carriers, and were associated with reduced t-PA levels, significant increases in *PAI-1* levels were seen between cases and controls, irrespective of the genotype. Summary/Conclusion. Whereas significant differences were seen in the distribution of some PAI-1 4G/4G and G[-844]A variants between cases and controls, yet their modest influence on *PAI-1* levels and activity (t-PA) suggests the contribution of other stroke-associated factors in modulating PAI-1 levels and activity.

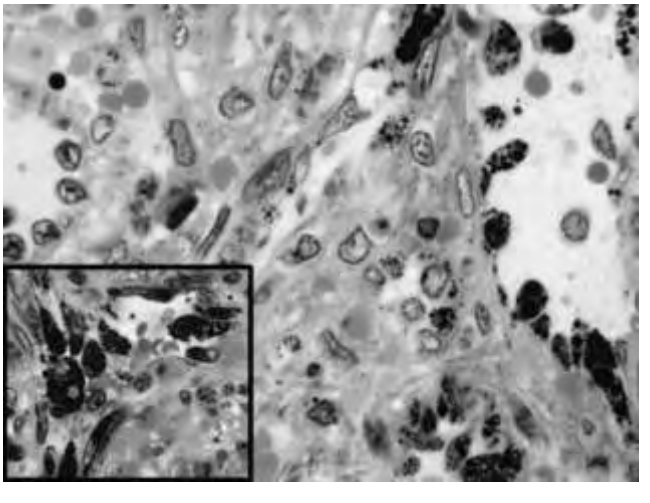
1097

VASCULAR AND SINUSOIDAL ENDOTHELIAL ACTIVATION, PROLIFERATION, DIFFERENTIATION AND ERYTHROPHAGOCYTOSIS: ULTRASTRUCTURAL FINDINGS ON A CASE OF AUTOIMMUNE LYMPHOPROLIFERATIVE SYNDROME (ALPS)

H. Sencer,[1,2] S. Gozdasoglu,[2] Z. Uysal,[2] T. Ileri,[2] F. Azik,[2] M. Ertem,[2] S. Erekul[2]

[1]*Ankara Universty, School of Medicine, Ankara, Turkey;* [2]*Ankara University School of Medicine, Ankara, Turkey*

Backgrounds. Since 1991, one of us (Sencer H) has reported that; 'vascular endothelial cells have reserved the capacity of stem cell and can activate, proliferate, and differentiate to other stromal and hemapoietic cells in health and diseases. Activated endothelial cells can migrate to the stroma or circulate in the vascular lumen as 'circulating progenitor endothelial cells (CEC/CPEC)' after plumping and detaching from basal lamina. Besides, viral replications and damages on erytrocytes were clearly demonstrated ultrastructurally by Sencer H in 1995. Aims. The aim is to provide morphological basis of functional modifications occurring in the disease. This is the first ultrastructural study on ALPS, to our knowledge. *Case Report and Methods.* The patient was healthy until the age of 6 months when he presented with disseminated vesicular skin lesions, generalized lymphadenopathy, hepatosplemegaly, tachycardia and was diagnosed with severe varicella zoster virus (VZV) infection. Coombs positive(IgG) hemolytic anemia, thrombocytopenia, elevated immunoglobulin levels and severe proteinuria were detected. CMV IgM and IgG were also found to be positive. At the age of 10-month CMV IgM and whole blood polimerase chain reactions analysis for CMV were negative. He presented with Evans syndrome symptoms and he was diagosed as ALPS after the detection of increased percentage of double negative T cell population in the peripheral blood. The patient underwent splenectomy at the age of 20 months because of refractory thrombocytopenia. Material for this study were obtained during splenectomy and performed EM preparation. Semi-thin sections were stained with toluidine blue-borax. Thin sections were contrasted with uranyl acetate/lead citrate and observed with JEOL100BEM.



Results. Red pulp was widespread, but white pulp wasn't distinctive with increased follicular hyperplasia and prominant marginal zone in the spleen. Increased fibrotic elements some of which related several arteries in plane of sections and plasmacytes were seen. Virus-like particles were observed. Activation and proliferation of vascular and sinusoidal (littoral) endothelial cells had occured. Some of them were committed to erythrophagocytosis which were became large and shuttle shape. Their cytoplasms were full with erytrocytes, erytrocyte fragments and/or phagocytic end-products. Erytrocytes probably damaged with viruses- were internalized by endothelial cells, but could not been digested totally. Both of the activated and phagocytic endothelial cells could detach from their original sites and move to the sinusoidal and/or vascular lumen. These could functionally be named circulating endothelial progenitor cells (CPEC) and circulating erythro-phagocytic endothelial cells (CEPEC) respectively. These were neither sinusoidal histiocytes, nor cordal macrophages classical. *Conclusions.* We suggest that the splenic endothelial cells have erythrophagocytic activity in ALPS. Viral replication on erythrocytes and/or endothelium may be causative agent. Endothelium should be most important key system in the health and diseases. Electron microscopy is useful to avoid misinterpretation.

1098

ENDOTHELIAL MICROPARTICLES AND MARKERS OF COPPER METABOLISM AS NOVEL INDICATORS OF ANGIOGENESIS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

L. Smolej,[1] D. Vokurkova,[2] C. Andrys,[2] D. Kakrdova,[2] J. Krejsek,[2] J. Maly[2]

[1]*Charles Univ.Hospital and Medical School, Hradec Kralove, Czech Republic;* [2]*Charles Univ. Hospital, Hradec Kralove, Czech Republic*

Backgrounds. Angiogenesis is currently considered an important process in biology of B-cell chronic lymphocytic leukemia (B-CLL). Copper is an important cofactor for some angiogenic factors. Elevated serum levels of copper (Cu) and its transport protein coeruloplasmin (CP) have been reported in patients with advanced cancers. Endothelial microparticles (EMPs) are fragments of endothelial cells which are produced during endothelial proliferation or damage and circulate in peripheral blood. Neither parameters of copper metabolism nor EMPs have been used so far to assess angiogenesis in B-CLL. *Aims.* To analyze serum concentrations of Cu and CP and quantitate EMPs in patients with B-CLL. Methods. We measured serum Cu and CP in 19 patients with B-CLL diagnosed according to NCI-WG criteria. Cu was measured using chromatography and CP by immunoturbidimetry. EMPs were analyzed in 20 B-CLL patients and 10 healthy donors using two-colour flow cytometry of platelet-poor plasma. CD105 (endoglin) and CD144 (VE-cadherin). CD41 was used as a platelet marker. *Results.* Cu and CP were detectable in all B-CLL patients. Both markers were in normal range (Cu: mean ± SD [standard deviation], 18.13±3.98 μmol/l, 95% CI [confidence interval] of mean, 16.21-20.05 μmol/l; CP: mean±SD, 0.294±0.062 g/L, 95% CI of mean, 0.264-0.324 g/L). Neither Cu nor CP were significantly different between B-CLL patients with stable (n=7) and progressive (n=12) disease (p=0.77 and 0.54, respectively). There was a statistically significant increase of CD41+/105+ microparticles (mean ± SD 142.8 ± 22.4/ul, 95% CI of mean, 95.8-189.7/μL) in B-CLL patients when compared to control group (mean ± SD [standard deviation], 60.8 ± 32.4 /ul, 95% CI of mean, 37.6-84.0 /ul; p=0.003). There was no significant difference between patients with. *Conclusions.* Our study is the first one to report measurement of endothelial microparticles and markers of copper metabolism as angiogenesis indicators in CLL. However, neither serum Cu nor CP were significantly elevated in B-CLL patients over controls. In addition, we did not observe differences in Cu or CP levels between patients with stable vs progressive disease. Furthemore, we found elevated numbers of CD41+/105+ (aggregates of platelets and EMPs) but not CD144+ or CD105+ EMPs in B-CLL patients. Larger study is clearly warranted to confirm these findings and perform a detailed statistical analysis including comparison with other angiogenic markers.

Funding: Supported by grant NR/8373-3 and research project MZO 00179906 from Ministry of Health of Czech Republic and by grant from League Against Cancer, Prague, Czech Republic.

1099

ELIMINATION OF IRON IN HEREDITARY HEMOCHROMATOSIS PATIENTS TREATED WITH ERYTHROCYTAPHERESIS

V. Rehacek

Charles University Hospital, Hradec Kralove, Czech Republic

Backgrounds. Hereditary hemochromatosis (HH) is an inherited, autosomal recessive disorder of iron metabolism that causes the body to absorb and store an excess amount of iron resulting in the progressive accumulation of iron in the liver, pancreas, heart, joints, and pituitary gland leading to potentially serious complications including cirrhosis of the liver, diabetes, and heart problems. The effective treatment is the regular whole blood removal which causes erythropoesis activation and leads to decrease of iron stores. Red cell apheresis is an optional method for removing of higher amount of erytrocytes in one session. The aim of this study was to evaluate the effectiveness of erythrocytapheresis in the treatment of HH. *Methods.* Repeated erythrocytapheresis were performed in 17 patients with diagnosis of HH (15 x C282Y homozygotes, 2x C282Y + H63D heterozygotes) using Haemonetics MCS 3p cell separator (protocol TAE) in which red cells were removed from patients in 2 - 5 cycles; plasma and buffy-coat were reinfused. Collection time, donor convenience, side effects and red cell yield were recorded and analysed. Samples for hematology and iron studies in patients were drawn, analysed and compared to baseline levels. *Results.* 276 (3 - 70) red cell apheresis in 17 patients (13 male, 4 female), age 49,9 (32 - 67), height 175,7cm (160 - 190), weight 82,8 kg (55 - 110), TBV 5186 mL (3627 - 6501). Procedure time was 32 - 87 min. Mean Hb level decreased from 141,7 g/L (115 - 155) before the procedure to 121,6 g/L (93 - 130). Ferritin values decreased from 1199 ng/mL (268 - 3998) to less than 25 ng/mL (7 - 23,9) in each of patients. The drop in ferritin level was 175 ng/mL (67 - 358) per month and 86 ng/ml (41 - 135) per one apheresis, respectively. *Conclusions.* Procedures were well tolerated by patients, no serious side effects were seen, 21 mild citrate reactions (7,6%) were noted. Red cell apheresis is an effective procedure of iron stores reduction in pacients with the hereditary hemochromatosis. Decrease of iron stores in pacients is individual and depends on many factors.

Funding: Supported by research project MZO 00179906

1100

THERAPEUTIC LEUKAPHERESIS EXPERIENCE OF A SINGLE CENTRE

A. Cunha,[1] M. Rosales,[2] S. Roncon,[2] A. Aguiar,[2] A. Carvalhais[2]

[1]*Centro Hospitalar de Vila Nova de Gaia, V N Gaia, Portugal;* [2]*Instituto Portugus de Oncologia, Porto, Portugal*

Therapeutic Leukapheresis (TL) is an option in the management of patients with hyperleucocytosis, especially associated with leukostatic symptoms. Nevertheless, its clinical and analytical benefit is not well documented in the literature. The aim of this study was to retrospectively analyse the TL performed in our Centre, between January 1998 and December 2005 and also to evaluate its efficacy and complications. During this period, 28 TL were performed in 15 patients (9 men/6 women), with a median age of 22 years (range 2-78), diagnosed with Acute Lymphoblastic Leukaemia (n=6), Acute Myeloblastic Leukaemia (n=7) and Chronic Myeloid Leukaemia (CML) (n=2). Most of the patients (n=14) initiated TL within one week after the diagnosis. One pediatric patient with CML and an initial white blood cells (WBC) count of 306×10^9/L did not have leukostatic symptoms. The others presented cerebral (lethargy, aphasia, dysarthria, altered vision, intracranial haemorrhage) and/or pulmonary (dry cough, respiratory distress and alveolar haemorrhage) manifestations. Each patient was treated with a median of 2 TL (1-4). Aphaereses were performed in a Cobe Spectra cell separator in the Intensive Care Unit. The mononuclear cells program (MNC) was selected in 20 procedures and the polymorphonuclear cells program (PMN) in the other 8 cases. A median of 3 blood volumes per TL was processed (1-4). An efficacy index (EI) was calculated in order to monitor the procedures: EI = (total collected WBC / total pre-aphaeresis patient WBC) x 100. The median pre-aphaeresis WBC count was 213×10^9/L (65-856), which had a corresponding median leukocrit of 8 ml/dL (2-26). The median EI of all TL was 20% (0-47) and when considering each program, the PMN had a median of 23% (16-47) and the MNC achieved 13% (0-30). The median WBC count, 1 hour and 24 hours after TL, was 174×10^9/L (45-650) and 173×10^9/L (109-491), respectively. Serious complications occurred in 4 patients leading to TL interruption. Those were: respiratory arrest, hypotension, respiratory failure and mucocutaneous haemorrhage; however no deaths occurred. Hypocalcaemia related side effects were observed in 13 patients, but promptly reverted with calcium gluconate administration. After TL, clinical improvement was observed in 6 patients who survived at least for one month. The remaining 9 patients, maintained or worsened their condition and all had an early death (<30 days). The overall survival rate at 6 months after TL was 40%. In summary, in this Centre, the majority of patients who underwent TL were critically ill. Even though, the survival rate was similar to the reported in the literature. The lack of immediate clinical improvement was a sign of a poor prognosis. The PMN program was found to be more effective than the MNC and the EI revealed an easily calculated and reliable indicator. Conclusions were limited due to the reduced number of patients in the study. It is important to find standard indicators to technically and clinically monitor the TL, in order to allow multicentric comparisons from the data available.

1101

RETROSPECTIVE ASSESSEMENT OF THE GLOBAL QUALITY OF LIFE OF PATIENTS WITH ACUTE MYELOID LEUKEMIA AFTER HSCT FROM NURSES PERSPECTIVES: FINDING FROM A CROSS-SECTIONAL AND RETROSPECTIVE STUDY.

L. Slovacek,[1] B. Slovackova,[2] L. Jebavy,[1] M. Blazek,[3] J. Horacek[1]

[1]*University of Defence, Hradec Kralove, Czech Republic;* [2]*Psychiatric Clinic, Hradec Kralove, Czech Republic;* [3]*Department of Hematology, Hradec Kralove, Czech Republic*

Backgrounds. The cross-sectional and retrospective study analyses the selected factors which influence global quality of life (QoL) of patients with acute myeloid leukemia (AML) after the hematopoietic stem cell transplantation (HSCT). *Aims.* to verify the applicability of the Czech version of an international generic European Quality of Life Questionnaire - Version EQ-5D for the evaluation of global QoL in patients with acute myeloid leukemia (AML) after HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove Czech Republic and to evaluate the global QoL in patients with AML after HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic, 3. to analyse selected demografic, healthly and social factors which influence global QoL in patients with AML after HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic. Patients and Methods. The total number of respondents after the transplantation from 2001 to 2003 was 19 and the return rate of questionnaires was 63% (12 respondents: 9 respondents with AML after autollogous HSCT, 3 respondents with AML after allogenous HSCT. HSCT. The mean age of patients with AML was 47,5 years old (range 27-68) and the male / female ratio was 1,17/1. The Czech version of an international generic EuroQol Questionnaire - Version EQ-5D was used. The influence of monitored factors (age, sex, education, merital status, polymorbidity, nicotinism, religion, type of HSCT and the time lapse from the HSCT) on global quality of life of patients was determined by means of dispersion analysis. Results. The above-mentioned factors proved statistically significant dependence of EQ-5D score and EQ-5D VAS on age (in both cases $p<0,01$), religion (in both cases $p<0,05$), nicotinism (in both cases $p<0,01$), education (in both cases $p<0,05$) and polymorbidity (in both cases $p<0,05$). *Conclusion*: EQ-5D score (dimensions of QoL) and EQ-5D VAS (a subjective health condition) significantly decrease with increasing age, religion, nicotinism, education and polymorbidity on patients with AML after HSCT. The global QoL of patients with AML after HSCT is high (mean EQ-5D score 75,1%, mean EQ-5D VAS 67,5%).

1102

A 5+5 YEAR EUROPEAN NON-INTERVENTIONAL SAFETY STUDY COMPARING ANAGRELIDE HYDROCHLORIDE (XAGRID) WITH OTHER CYTOREDUCTIVE TREATMENTS IN AT-RISK ESSENTIAL THROMBOCYTHEMIA SUBJECTS

G. Birgegård,[1] J.Y. Cahn,[2] M. Greisshammer,[3] L. Gugliotta,[4] C. Besses,[5] C. Harrison[6]

[1]*University Hospital, Uppsala, Sweden;* [2]*Universitaire de Grenoble, Grenoble, France;* [3]*Med. Universitatsklinik Ulm, Ulm, Germany;* [4]*Arcispedale S. Maria Nuova, Reggio Emilia, Italy;* [5]*H. Del Mar, Barcelona, Spain;* [6]*St Thomas' Hospital, London, United Kingdom*

Backgrounds. Long-term data supporting the use of the different cytoreductive agents in the management of elevated platelet counts remains sparse, particularly when analysing long-term safety. Anagrelide is a selective, non-cytotoxic platelet reducing agent that has been used exten-

sively worldwide. It has been licensed since 1997 for use in the US and since 2005 in Europe. *Aims.* This post-registration, non-interventional study (EXELS: Evaluation of Xagrid Efficacy and Long-term Safety) was started on the initiative of the EMEA as a long term safety and efficacy study in a cohort of at-risk essential thrombocythaemia (ET) subjects exposed to anagrelide or other cytoreductive treatments. The primary study objective is to continuously monitor safety and pregnancy outcomes. Secondary objectives are to assess efficacy (platelet count and number of thrombohemorrhagic events) and drug utilization (including drug dose and duration of exposure). *Methods.* This 5 + 5 year European non-interventional study, which is being led by a steering committee of ET experts and continuously evaluated by an independent Data and Safety Monitoring Board, will enrol a minimum of 1000 at-risk ET subjects receiving anagrelide and up to 3000 at-risk ET subjects receiving other cytoreductive therapies. All cytoreductive agents must be prescribed in accordance with the appropriate product information. Subjects may be newly diagnosed or continuing their existing medication for the treatment of ET. Concomitant medication use is at the discretion of the investigator. Data will be collected without any interference with the treatment choice of physicians and will be captured electronically by use of a web-based registry utilizing electronic case report forms. An initial 5-year study period will focus on the collection of data related to a number of pre-defined events. These include complications of the disease (thromboembolic and hemorrhagic events as well as transformation) and possible toxic complications (congestive cardiac failure, cardiomyopathy, severe mucocutaneous disorders, pulmonary hypertension, pulmonary fibrosis/interstitial pneumonia, pancreatitis, rhabdomyolysis/myalgia and non-haematological malignancy). Death, as well as the incidence of serious adverse events related to current ET therapy will be recorded. Events will be evaluated by an independent Event Validation Panel. If required, based on review of data from the initial 5-year phase, data will be collected during a second 5-year study period to assess selected pre-defined events including pregnancy (and progeny) outcomes, serious adverse events related to the current ET therapy, and other events as defined by the steering committee. *Results.* Study recruitment began on 30 May 2005. To date, 364 subjects have been recruited with the expected ratio of 1:2 between anagrelide and other platelet reducing therapy. *Summary/Conclusions.* This ongoing non-interventional study is expected to provide quality data from a large cohort of at risk ET patients, assessing the long-term safety of agents used to reduce platelet levels in patients with ET as well as data regarding the protective power against disease complications.

1103

THROMBOTIC AND HEMORRHAGIC EVENTS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA (ET) DURING THERAPY WITH INTERFERON -α OR ANAGRELIDE

E. Plata, S.I. Kokoris, T.P. Vassilakopoulos, S. Sachanas, P. Tsaftaridis, E.M. Dimitriadou, Z. Galanis, M.P. Siakantaris, N.-A. Viniou, G.A. Pangalis,

University of Athens, ATHENS, Greece

Backgrounds. Essential thrombocythemia (ET) is a clonal myeloproliferative disease characterised by sustained thrombocytosis and increased number of megakaryocytes in the bone marrow. The most severe complications and the principal causes of death in these patients include thrombosis, haemorrhage and progression to myelofibrosis, or acute myelogenous leukemia. Several agents have been reported to control the disease. Two of the most frequently administered are interferon and anagrelide. *Aim*. To retrospectively evaluate the incidence of thrombotic or hemorrhagic events in patients with ET during therapy with interferon-α or anagrelide. *Methods.* In a cohort of 195 patients with ET, who are followed up in our center during the last twenty years, we recorded 24 patients treated with IFN-α (group A) and 37 patients treated with anagrelide (group B). All other patients were treated with low dose aspirin, hydroxyurea, or busulfan. Group A included 8 males and 16 females, with a median age of 42.5 years (range 22-74). Group B included 15 males and 22 females with a median age of 41 years (range 14-70). At diagnosis, 4 patients in Group A presented with thrombotic episodes vs 3 patients with thrombotic and 1 with hemorrhagic episode in Group B. Interferon was administered at a dose of 3 MU, subcutaneously, three times per week and anagrelide at a dose range of 1-3 mg per day. The goal of the treatment was to lower the platelet count below 500×10^9/L. *Results.* All patients achieved the proposed goal of treatment. One thrombotic or hemorrhagic complication in Group A vs 6 in Group B were recorded during therapy. These complications were: in Group A an hemorrhagic cerebral episode and in Group B heart attack and mesenteric vein thrombosis in the same patient, femoral vein thrombosis, transient ischemic cerebral episode, erythromelalgia and severe nasal haemorrhage. None of these episodes were fatal and all patients recovered. The median follow up was 68.5 months (range 4 -196) under interferon-α, and 32 months (range 1-94) under anagrelide. In Group A we did not observe any thrombotic event, while we recorded one hemorrhagic event for a rate of 0.6% per year after 155 persons-years of follow up. In Group B we observed 1 hemorrhagic event for a rate of 0.9% per year after 107 persons-years of follow up, while we recorded 5 thrombotic events for a rate of 4.7% per year. *Conclusions.* We observed a higher incidence of thrombohemorrhagic, especially thombotic events in the anagrelide group. This has been observed in other clinical studies with anagrelide as well. Despite the fact that this is a retrospective, no randomised study with a small number of patients, these results made us more sceptical regarding the use of anagrelide in patients with ET, as a first-line treatment.

1104

CHRONIC NEUTROPHILIC LEUKAEMIA WITH AN ASSOCIATED V617F JAK2 MUTATION: EVIDENCE OF MONOCLONAL ORIGIN OF T LYMPHOCYTE AND GRANULOCYTE LINEAGES

M.J. Percy,[1] D.P. McLornan,[1] M.F. McMullin[2]

[1]*Belfast City Hosptial, Belfast, United Kingdom;* [2]*Queen's University, Belfast, United Kingdom*

Backgrounds. Chronic Neutrophilic Leukaemia (CNL) belongs to the atypical myeloproliferative group of disorders (MPD) and is a rare disease entity. It is characterised by a short term survival and transformation to acute leukaemia. Previously, we have described an individual with CNL who has survived more than 103 months with the disease. Subsequently, the patient has exhibited evidence of morphological transformation to a refractory anaemia with excess blasts (RAEB) 2 with clonal evolution as indicated by the recent detection of a cell population with chromosome 7 monosomy. Screening for the V617F Janus Kinase (JAK) 2 mutation, which is highly prevalent in classical MPDs and in particular polycythaemia vera, the patient proved to be homozygous for the mutation in the granulocyte and macrophage lineages [*McLornan et al (2005) Haematologica 90:1696*]. There is evidence to suggest that in the case of CNL both granulocytes and T cells can be derived from the same clone [*Bohm et al (2003) J Clin Pathol 56:292*]. Consequently, if the granulocyte lineage was positive for the *V617F JAK2* mutation then the T lymphocytes would also be positive. *Aims.* To investigate whether the T and B cells are positive for the *V617F JAK2* mutation and derived from the same clone as the granulocytes in a patient with advancing CNL. *Methods.* B lymphocytes were isolated by CD19+ immunomagnetic selection from the total lymphocyte fraction prepared from density gradient Ficoll separated whole blood. The remaining cells post immunomagnetic selection, which were T lymphocytes and natural killer (NK) cells, were retained. DNA was prepared from both B and T lymphocyte cell fractions and PCR-direct sequencing was performed. *Results.* Screening for the V697F mutation by sequencing indicated the B lymphocytes exhibited wild type *JAK2* only. Conversely, sequencing the cellular fraction containing isolated T lymphocytes and NK cells indicated clearly they displayed heterozygosity for the*V617F JAK2* mutation. *Summary.* Screening for the *V617F JAK2* mutation demonstrated the mutation was absent in the B lymphocytes but was present in the T lymphocyte lineage. Previously, we detected this same mutation in the homozygous state in granulocyte and macrophage lineages but it was absent in cells derived from the buccal cavity and hair follicles, thus confirming that the mutation was acquired. Since the T lymphocytes and granulocytes were positive for the *V617F JAK2* mutation it would suggest that both lineages were derived from the same neoplastic clone in this case of CNL. Thus our results confirm the previous observations reported by Bohm *et al*. (2003) in 4 CNL patients using Humara assays, which indicated monoclonality of T and granulocyte lineages. Finally, it remains to be established what exact role the *V617F JAK2* mutation, which gives cells a proliferative advantage, plays in the pathogenesis and prognosis of rare atypical MPDs such as CNL.

1105

SYSTEMIC MASTOCYTOSIS. AN ITALIAN MULTICENTRIC RETROSPECTIVE SURVEY

L. Pagano, C.G. Valentini, M. Caira, M.T. Van Lint, P. Musto, A. Candoni, G. Martinelli, M. Rondoni, B. Allione, C. Cattaneo, L. Marbello, C. Caramatti, E.M. Pogliani, M.E. Mitra, L. Fianchi, G. Leone

Hematology Insitute, Rome, Italy

Background/Aims. To evaluate clinical and molecular features, and outcome of patients (pts) with Systemic Mastocytosis (SM). *Methods.* A retrospective revision of 26 cases of SM, diagnosed in 11 Italian Hematology Divisions between 1995 and 2006. *Results.* 26 new cases of SM were collected and classified according to the WHO criteria: Mast Cell Leukemia in 12 pts, Aggressive SM in 10 and Indolent SM in 3; the remaining one had SM with associated clonal non-mast cell-lineage hematologic disease (AML). Skin was the principal extramedullary organ involved by uncontrolled proliferations of MC (17 pts) followed by spleen (13), liver (12), and cardiovascular system (12). In 61% of cases constitutional symptoms (fatigue, itchiness and abdominal pain) were present. Molecular biology studies were performed in 18 pts: 12 showed the c-kit point mutation D816V; in 3 pts additional gene defects and karyotype abnormalities were recognized. Treatments were very heterogeneous, and the same patient could have received different therapies after failure of the previous one. Seven patients were not initially treated: 5 maintained a stable disease, while 2 had a progressive clinical course. Imatinib (400 mg/day) was used in 15 pts (10 as first line therapy, 4 and 1 as second and third line respectively); c-kit mutation was present in 9 of these 15 pts. A partial response was obtained in only one of them (response rate 11%); among the remaining 6 patients without the c-kit mutation, partial or complete remissions were obtained in 2 and 1 pts respectively (33% and 17%). Interferon-α (3x3 milion units s.c. weekly) was employed in 6 patients (3 as first line therapy, 2 as second and 1 as third line): a partial remission was achieved in one case only (17%). 2 CDA (0.14 mg/kg) was administered in 3 pts (1 as first, 1 as second and 1 as third line therapy) registering a partial remission in all of them. One patient performed only radiotherapy and achieved a pure clinic major response. In 4 cases were used other chemotherapies (in 2 pts as first and in other 2 as second therapy) with no response; and 3 received steroid therapy, not in association with other drugs, obtaining in 1 case a partial response. Two patients underwent stem cell transplantation as second and fourth line respectively, obtaining both a complete remission. Two pts (8%) who had received conventional chemotherapy only, died for mastocytosis; a third patient in complete remission of disease died for accidental causes. The 10-years survival rate is about 88%. *Summary/Conclusions.* Our results suggest that SM is a very rare disease, but although severe and life-threatening mediator-related symptoms, the mortality is low. D816V c-kit mutation is associated with relative resistance against Imatinib. Among purine analogues, 2-CDA has shown interesting clinical response, while INF has not offered any benefits although the similarity between SM and myeloproliferative diseases. Because of the rarity of this disease, an effective standard of care is lacking: for this reason more data are needed to find new and successful therapeutic strategies, such as other tyrosine kinase inhibitors.

1106

COMPARISON OF THE RESULTS FOR THE JAK2 V617F MUTATION DETECTION BY TWO METHODS; ALLELE SPECIFIC PCR AND RESTRICTION DIGESTION ASSAY ON POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA DNA SAMPLES

T.P. Pajic,[1] J. Vuckovic,[2] L. Goriek,[1] C. Appleton,[3] U. Mlakar[1]

[1]*University Medical Centre Ljubljana, Ljubljana, Slovenia;* [2]*General Hospital Celje, Celje, Slovenia;* [3]*Hospital de Santa Maria, Lisboa, Portugal*

JAK2 V617F is a clonal acquired mutation found in the majority of patients with polycythemia vera (PV), and a significant number of patients with essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF). The incidence of this mutation ranging from 65% to 97% in PRV patients, from 25% to 57% in ET patients and about 50% in MMM depending on the study. These variations in percentage of patients involved is likely due to the criteria used for diagnosis and also the sensitivity of the assay used to detect this mutation. Allele specific PCR and restriction digestion assay by enzyme BsaXI are usable tehniques for its detection. The aim of this study was to compare the results for the JAK2 V617F mutation detection by two frequentlly used techniques on DNK of granulocytes of the PV and ET patients. Furthermore, the results were compared to that published in the literature. The diagnosis of ET and PV was established followed World Health Organization classification. 19 patients with PRV and 42 patients with ET were inculded in the study. EDTA peripheral blood was drawn and used for the isolation of the granulocytes by Ficoll density centrifugatuion followed by dextran sedimentation. DNA was isolated from granulocytes by High Pure PCR Template Reagent kit from Roche The allele specific PCR was carried out as described in Baxter EJ *et al.* (*Lancet 2005; 365:1054-61*). Restriction digestion assay by enzyme BsaXI was carried out by test, designed by InVivoScribe Technologies (San Diego, USA). The PCR and resctriction digestion products were visualized after agarose gel electrophoresis by ethidium bromide staining. The concordance between this two methods was 100%. The percentage of positivity for JAK2 V617Fmutation in ET and PV patients were 50 (21/42) and 95 (18/19), respectively. We conclude that both methods are appropriate for the routine detection of JAK2 V617F mutation on DNA samples from granulocytes from peripheral blood in ET and PV patients. In our hands the sensitivity of this two methods was the same. The percentage of positivity for JAK2 V617F mutation in ET and PV patients was similar of that published in the literature and was in the upper part of the range.

1107

MICROVESSELS DENSITY (MVD) AND VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IMMUNOHISTOCHEMICAL EXPRESSION IN PH(-) CHRONIC MYELOPROLIFERATIVE DISORDERS (CMPDS)

C. Vener,[1] U. Gianelli,[2] A. Moro,[2] P. Rafaniello Raviele,[2] R. Calori,[1] A. Iurlo,[3] F. Radaelli,[3] G. Lambertenghi Deliliers[1]

[1]*Ematologia Centro Trapianti di Midollo, Milano, Italy;* [2]*Anatomia Patologica, Osp.San Paolo, Milano, Italy;* [3]*Ematologia* [2]*, IRCCS, Milano, Italy*

There is increasing evidence that neovascularization may play an important role in haematological malignancies and in particular in lymphomas, acute leukaemias and myelodysplastic syndromes. However, few studies have been performed in order to evaluate this phenomenon in Ph(-) CMPDs. Increased angiogenesis in chronic idiopatic myelofibrosis (CIMF) and high serum concentration levels of VEGF, the most potent direct-acting angiogenic factor, in CMPDs were reported. Recently, an increased immunohistochemical espression of VEGF was demonstrated in CMPDs. A new classification of chronic CMPDs was worked out by the WHO, which highlighted the importance of bone marrow biopsy (BMB) in differential diagnosis and in the evaluation of myelofibrosis. In addition to standard therapy, new therapeutic ways of approaching and directly targeting endothelial cells or VEGF have been experimented in CMPDs, with variable results. The aim of this research was to examine the MVD and VEGF immunohistochemical expression in the different categories of Ph(-) CMPDs, according to the new WHO classification. We examined the BMBs of 90 CMPDs patients, classified according to the WHO classification. In particular, there were 30 cases of essential thrombocythaemia (ET), 30 of CIMF (10 CIMF-0, 10 CIMF-1 and 10 CIMF-2+3 sec CCGM - Haematologica 2005) and 30 of polycythaemia vera (PV) (20 polycythaemic phase and 10 post-polycythaemic myelofibrosis); we also analyzed 20 non-pathologic BMBs as normal controls. MVD analysis was performed according to the hot-spot methods, using an anti-CD34 antibody. The VEGF immunohistochemical expression was expressed as VEGF index, according to the mathematical formula [VEGF(i) = VEGF(+) x BMB cellularity/100]. All statistical tests were performed at the 5% significance level ($p<0.05$) (Anova oneway). Hot-spot MVD and VEGF(i) immunohistochemical results are described in Table 1.

Table 1. Hot-spot MVD and VEGF(i) immunohistochemical results.

	Vessels Medium N. ± SD (range)	*VEGF (i) ± SD (range)*
Controls	7,5±3,6 (4,6-16,3)	0,08±0,009 (0,01-0,15)
ET	10,1±4,4 (3,3-19,6)	0,12±0,05 (0,05-0,28)
CIMF-0	21,5±3,8 (15-26)	0,20±0,09 (0,12-0,42)
CIMF-1	29,3±5,4 (17,6-38,3)	0,36±0,18 (0,12-0,64)
CIMF 2+3	27,3±6,3 (20-44,6)	0,26±0,12 (0,01-0,42)
PV	17,3±7,4 (5,6-33,6)	0,21±0,15 (0,09-0,66)
MF post-PV	31,9±7,3 (15-40)	0,49±0,19 (0,05-0,64)

There is no difference in MVD and VEGF(i) expression between ET and control group. Moreover MDV and VEGF(i) proved to be much

higher in CIMF and PV than in the control group. MVD and VEGF(i) in fibrotic CIMF (CIMF-2+3) have been demonstrated statistically different from MDV and VEGF in myelofibrosis post-PV. Our analysis identified significant biological differences between the various types of myelofibrosis and could serve as a rationale guide in the antiangiogenetic therapy.

1108

JAK2V617F, PRV-1 EXPRESSION AND ENDOGENOUS ERYTHROID COLONIES GROWTH IN PATIENTS WITH POLYCYTHEMIA VERA

F. Pompetti,[1] A. Spadano,[2] I. Villanova,[3] A. Mennucci,[4] R. Russo,[4] R. Malizia,[5] A. Dragani,[5] T. Bonfini,[3] G. Fioritoni,[2] A. Iacone[6]

[1]*Civil Hospital/Hemat. Mol. Biol. Lab., Pescara, Italy;* [2]*Dep.Hemat/Div.Clinic.Hemat/C.Hospital, Pescara, Italy;* [3]*Dep.Transf.Med/Cell Cult. Lab./C.Hospital, Pescara, Italy;* [4]*Dep.Transf.Med/Hem.Mol. Biol. Lab/C.Hospit, Pescara, Italy;* [5]*Dep.Hem/Serv.Hemost.Throb/C.Hospital, Pescara, Italy;* [6]*Dep.Transfus. Med./C. Hospital, Pescara, Italy*

Background. Polycythemia vera (PV) is a chronic myeloprolipherative disorder (MPD) characterized by a primary increase of red cell mass. One of the WHO diagnostic criteria for PV is the *in vitro* endogenous erythroid colonies (EEC) formation. Some molecular alterations have been associated to MPD, and currently the most indicative molecular markers are the overexpression of *PRV-1* and the genomic mutation *JAK2V617F*. Both these alterations have been found in the majority of PV cases and in particular *JAK2* has been associated with the ability to form EECs, and seems to have a causal or a strongly contributory role in the pathogenesis of the MPDs and in particular of this primary erythrocytosis. Furthermore it has been suggested an allele dose-dependent association that links *JAK2V617F* and expression of PRV-1. *Aims*. To evaluate the association between EECs formation and the molecular alterations considered, we analyzed 21 cases of PV for EECs, *JAK2V617F* and PRV-1 expression. *Methods*. *JAK2V617F* was performed by allele-specific amplification, PRV-1 expression was normalized on GAPDH expression, analyzed with the ΔCt method and expressed as relative quantification (RQ); EECs were detected on methylcellulose-based medium with and without erythropoyetin addition. *Results*. *JAK2V617F* was found in 17/21 (81%), and 4 of the *JAK2V617F* -positive cases presented only the mutated allele. All the patients analyzed showed EEC growth. PRV-1 expression was evaluated in 14 patients at the diagnosis and in 7 patients under hydroxyurea administration; overexpression resulted in 15/15 and 3/7 (43%) patients, respectively. The RQ mean in the *JAK2V617F* -negative, heterozigous *JAK2V617F*-positive and homozigous *JAK2V617F* -positive groups of untreated patients resulted 3.7 (1.2-10.4), 10.95 (3.6-23) and 12 (1.2-24), respectively. A control group of 7 patients with secondary erythrocytosis was also analyzed and resulted negative to molecular and functional markers. Statistical analysis showed the following Results. a significant correlation between *JAK2V617F* mutation and EECs (p=0.007, R=0.63), no statistically significant but positive correlation (p=0.09, R=0.6) between *JAK2V617F* and PRV-1 overexpression and a complete agreement between *PRV-1* overexpression and EECs results. Conclusions - Our study agrees with previous reports except for the association between *PRV-1* overexpression and *JAK2V617F*, but this result could depend on the small number of cases enrolled. In addition, although it could be possible an allele dose-depending effect on PRV-1 expression by JAK2 mutation, the RQ values inside each single group show a large variability, indicative of the high heterogeneity of the *JAK2V617F* -positive patients. Nevertheless, the absence of mutation in a group of patients with PV that presented EECs and PRV-1 overexpression strongly suggest the presence of other unknown factors involved in the pathogenesis of this disease. Our study suggests that EEC, PRV-1 expression and *JAK2V617F* mutation are useful biological markers to discriminate primary from secondary erytrocytosis. Nevertheless, these prelimary data should be confirmed in larger and prospective studies of newly diagnosed and treated patients.

1109

JAK2V617F MUTATION, PRV-1 OVEREXPRESSION AND EECS IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

F. Pompetti,[1] A. Spadano,[2] I. Villanova,[3] A. Mennucci,[4] R. Russo,[4] O. Iuliani,[5] A. Dragani,[5] T. Bonfini,[3] G. Fioritoni,[2] A. Iacone[6]

[1]*Civil Hospital/ Hemat.Mol.Biol.Lab., Pescara, Italy;* [2]*Dep.Hemat/Div.Clinic.Hemat/C.Hospital, Pescara, Italy;* [3]*Dep.Transf.Med/Cell Cult.Lab./C.Hospital, Pescara, Italy;* [4]*Dep.Transf.Med/Hem.Mol.Biol.Lab/C.Hospit, Pescara, Italy;* [5]*Dep.Hem/Serv.Hemost.Throb/C.Hospital, Pescara, Italy;* [6]*Dep.Transfus. Med./C. Hospital, Pescara, Italy*

Background. JAK2V617F is a genomic mutation associated to myeloprolipherative disorders such as polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis, that has been highly correlated with the ability to form endogenous erythroid colonies (EEC) and with PRV-1 overexpression. Nevertheless JAK2V617F mutation and PRV-1 overexpression are present in the majority of PV but in a percentage significally lower of patients with ET, suggesting a different role or effect of these molecular alterations in the different chronic myeloproliferative disorders. In particular the presence of JAK2V617F mutation is associated in ET patients to multiple clinical features resembling PV, suggesting the need of a new diagnostic classification based on the genotipic profiles. *Aims*. In the attempt to evaluate the incidence of the genetic alterations described, and the association to hematological features, we analyzed JAK2V617F, PRV-1 expression and EEC growth in a cohort of 46 patients with essential thrombocythemia. *Methods*, JAK2V617F was performed by allele-specific amplification, PRV-1 expression was normalized on GAPDH expression and analyzed with the ΔCt method, the EECs were detected on methylcellulose-based medium with and without erythropoietin. *Results*. JAK2V617F mutation was present in 11/46 (24%) cases; PRV-1 was overexpressed in 12/43 (27.9%) cases, in particular 7/27 untreated patients (25.9%) and 5/16 (31.3%) patients in therapy; 30/39 (76.9%) cases showed the ability to form EEC. The statistical evaluation of the data showed a significant correlation between JAK2V617F and EEC growth (p=0.04, R=0.33), but the correlation between JAK2V617F and PRV-1 overexpression was not significant. *Conclusions*. Our study seems to be in accordance with previous reports regarding the incidence of the molecular alterations found, but the correlation was statistically significant only between JAK2V617F and EEC. In fact, considering PRV-1 overexpression, neither correlation was statistically significant, nor was found any allele dose-depending effect on PRV-1 expression by JAK2V617F mutation. Finally, we did not find a difference between cases analyzed at the diagnosis and cases that were in therapy. Considering hematological parameters such as white blood cells and platelet counts, and hemoglobin level, the subgroup JAK2V617F-positive did not display higher values than JAK2V617F-negative patients. Thus we did not find distinctive hematologic characteristics that differentiate ET JAK2V617F-positive or negative.

1110

INCREASED ANGIOGENESIS IN CHRONIC IDIOPATHIC MYELOFIBROSIS: VEGF AS KEY ANGIOGENIC FACTOR

M.S. Steurer,[1] F. Augustin,[1] D. Fong,[1] S. Heiss,[1] K. Strasser-Weippl,[2] G. Gastl,[1] A. Tzankov,[1]

[1]*Innsbruck Medical University, INNSBRUCK, Austria;* [2]*Wilhelminenspital, Vienna, Austria*

Backgrounds. Recent studies suggest that increased angiogenesis is implicated in the pathogenesis of chronic idiopathic myelofibrosis (CIMF). Its impact on prognosis, however, is still la matter of debate. Vascular Endothelial Growth Factor (VEGF) is a potent stimulator of angiogenesis which is expressed in virtually all types of malignancies. We therefore hypothesised that VEGF may also play a role as angiogenic mediator in CIMF. *Aims.* The purpose of this study was to assess the prognostic value of bone marrow angiogenesis and its correlation with clinical parameters and cytogenetics in patients with newly diagnosed, untreated CIMF. Moreover, we aimed to investigate the expression of VEGF in bone marrow of CIMF. *Methods.* Between 1990 and 2001 all patients who were diagnosed as having CIMF at our center and for whom adequate bone marrow sections and clinical data were available, were deemed eligible. Each case was re-classified according to WHO-criteria. As a surrogate marker for angiogenesis we used microvessel density (MVD) as assessed by CD34 staining on paraffin-embedded trephine biopsy specimens. VEGF expression was examined by standard

immunohistochemical technique. The cytogenetic phenotype was determined by FISH on de-paraffinized bone-marrow sections. Appropriate summary statistics were used for comparisons between groups; survival was calculated using Kaplan-Meier estimates. Parameters found to be of prognostic significance in univariate analysis were verified in a multivariate Cox regression model. *Results.* Fifty-five patients were included in this retrospective single-center study. Clinical, cytogenetic and immunohistochemical data were available for all patients. With a median follow-up of 52,4 months (range 1 - 142 months), the median overall survival of the study cohort was 76,8 months. With a median MVD of 43 per 0.747 mm^2 field (range 6-96) CIMF patients displayed a significantly higher degree of bone marrow microvessel density than age-matched controls (n=10; median MVD=19, range 4'32; $p<0.001$). In fact, 85% of CIMF patients displayed an elevated MVD compared to normal controls. MVD was elevated significantly at all CIMF stages ($p<0.001$) with equal distribution between the various degrees of fibrosis (MF 0 - 3). Accordingly, VEGF expression was significantly higher in CIMF (median 12 cells per 0.747 mm^2 field) compared to normal controls (median 1.4 cells per 0.747 mm^2 field; $p=0.01$) and correlated with MVD ($p=0.001$). However, we found no correlations of MVD or VEGF expression with cytogenetics and clinical outcome, respectively. *Conclusions.* Our study confirms that bone marrow angiogenesis is increased in CIMF. In parallel, we found significantly elevated VEGF expression suggesting VEGF signalling to play a pathogenetic role and representing a potential therapeutic target in CIMF.

1111

JAK2-V617F MUTATIONAL ANALYSIS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

M. Speletas,[1] E. Katodritou,[1] E. Mandala,[2] C. Daiou[1], E. Poumpouridou,[3] C. Tsioni,[2] S. Efraimifou,[1] E. Papadakis[1], G. Papaioannou,[1] A. Kioumi,[1] K. Magiannis,[4] M. Papadopoulou,[5] K. Ritis,[6] I. Korantzis[1]

[1]*Papageorgiou General Hospital, Thessaloniki, Greece;* [2]*Aristotle University of Thessaloniki, Thessaloniki, Greece;* [3]*Saint Loukas Clinic, Thessaloniki, Greece;* [4]*General Hospital of Grevena, Grevena, Greece;* [5]*General Hospital of Katerini, Katerini, Greece;* [6]*Democritus University of Thrace, Alexandroupolis, Greece*

Backgrounds. An acquired mutation in Janus kinase 2 (JAK2) gene (characterized by a valine-to-phenylalanine substitution at position 617 [V617F] in the JH2 domain) has been recently described in the majority of patients with myeloproliferative disorders (MPDs). This mutation is associated with constitutive phosphorylation of JAK2 and its downstream effectors as well as induction of erythropoietin hypersensitivity in cell lines. However, its precise role remains to be determined. The aim of this study was to estimate the prevalence of the JAK2-V617F mutation, as well as the clinical and laboratory findings in patients with MPDs carrying the mutation. *Materials and Methods.* One-hundred and forty-two patients (M/F: 72/70, mean age: 59,8 years, range: 24-88) suffered from essential thrombocythemia (ET, 94), polycythemia vera (PRV, 39) and idiopathic myelofibrosis (IF, 9) were selected retrospectively from outpatient clinic between January 2000 and February 2006. Forty-five patients (31,7%) had at least one confirmed arterial and/or venous thrombotic episode either at diagnosis (mainly, as well 1-2 years ago) or during the follow-up period (mean: 38,8 months, range: 2-171). Genomic DNA was extracted from bone marrow aspirates or peripheral blood using standard protocol. The JAK2-V617F mutation was detected using both allele specific PCR and PCR-RFLP assay. Variables analyzed included age, gender, survival, thrombotic events, WBC, Ht, Hb, PLT, Epo, LDH, and the presence of splenomegaly, hepatomegaly and anticardiolipin antibodies both at diagnosis and during the follow-up period. Statistical analysis was performed by the SPSS software. *Results.* One-hundred and three patients exhibited JAK2-V617 mutational activation (33 of 39 with PRV, 84,6%; 63 of 94 with ET, 67,02%; 7 of 9 with IF). Interestingly, the patients carrying the JAK2-V617F mutation were older at diagnosis (61,3 vs 55,8, $p=0,026$), displayed lower Epo levels (8 vs 19,6, $p=0,001$), higher Ht (45,6 vs 42,1, $p=0,018$) and Hb values (15,1 vs 13,8, $p=0,021$), and presented more often with thrombotic events (35,9% vs 20,5%, $p=0,079$) and splenomegaly (32,03% vs 17,9%, $p=0,097$). In multivariate regression analysis, Epo levels and the presence of thrombotic events were independent variables correlated with the presence of mutation ($p=0,004$ and $p=0,038$, respectively). Moreover, 4 out of 5 patients who exhibited progression of the disease (3 with ET to IF and two with PRV to IF and AML, respectively) displayed the mutation, both before and after the deterioration of the disease. *Conclusion.* MPDs with JAK2-V617F mutation may prove to be a different disease entity than MPDs without JAK2-V617F mutation, with distinct clinical and laboratory findings.

1112

ROSAI-DORFMAN DISEASE (SHML) WITH NODAL AND MULTIPLE EXTRANODAL INVOLVEMENT COMPLICATED WITH AUTOIMMUNE HEMOLYTIC ANEMIA - A CASE REPORT

A.M. Petrescu,[1] M. Serban,[1] D. Herman,[2] A. Plesa,[2] U. Lohrs[3]

[1]*University of Medicine and Pharmacy, Timisoara, Romania;* [2]*County Hospital, Timisoara, Romania;* [3]*Patologisches Institut, München, Germany*

Background. Rosai-Dorfman disease or Sinus Histiocytosis with Massive Lymphadenopathy (SHML) described by Rosai and Dorfman in 1969, is a rare disorder (423 cases in the SHML registry) of unknown etiology, characterized by a nonmalignant proliferation of distinctive histiocytic/phagocytic cells within lymph node sinuses and lymphatics in extranodal sites (30%-40% of cases). *Aims*. Presentation of a patient with a severe form of SHML with nodal and multiple extranodal involvement (skin, upper respiratory tract, parotid gland, thymus), complicated with autoimmune hemolytic anemia. *Methods*. A gipsy male patient, one year old, was admitted with high fever, night sweats, asthenia, and loss of weight, inspiratory stridor, massive painless cervical and submandibular lymphadenopathy, parotid gland swelling, moderate hepatomegaly and splenomegaly. Extensive investigations were performed: imagistic, hematological, biochemical, immunological, serological, bacteriological, histopathological. *Results*. On examination, the patient was pale, moderately undernourished, had pronounced inspiratory dyspnea with stridor and dysphonia; the cervical, and submandibular lymph nodes were grossly enlarged bilaterally, 3-4 cm, of rubbery consistency, nontender. The parotid glands were enlarged. The x-Ray and CT scan also showed mediastinal and hillar lymphadenopathy and thymus enlargement. In evolution the patient developed a frank superior mediastinal syndrome, the axillary, inguinal and retroperitoneal lymph nodes were involved, and the hepatosplenomegaly progressed. The skin lesions located on the eyelids and periorbitar were nodules, and those on the thorax and limbs, pruritic erythematous papules. Biological investigations: moderate anemia, complicated than with Coombs positive anemia necessitating repeated transfusions, leukocytosis with neutrophil predominance, bone marrow with granulocytic hyperplasia; elevated ESR; high levels of γ globulins, IgG, fibrinogen, triglycerides, and ferritin; markedly decreased number of CD4+ cells and CD4+/CD8+ ratio, normal number of NK cells; serologic markers for EBV, HHV, CMV, HIV were negative; rheumatoid factor and antinuclear antibodies negative. Repeated lymph nodes and skin lesions biopsies were performed. The histopathological investigation failed to recognize SHML, giving different interpretations (Hodgkin disease, non-Hodgkin lymphoma, reactive lymphadenopathy); finally, the recognition of sinus histiocytosis and the hallmark of the SHML histiocyte, the lymphophagocytosis (emperipolesis), completed with immunohistologic investigation (histiocytes CD68+, S 100 protein+, CD1a-) confirmed the diagnosis. The treatment with prednisone 60 mg/m^2 for two months was efficient but the clinical and biological symptoms relapsed one month after. The interferon treatment was totally inefficient. Taking into account the severity of the disease, the treatment was continued with dexamethasone, etoposide and cyclosporine, for 52 weeks (HLH-94 protocol). The response was very good, with complete recovery maintained 32 months after completing the treatment. Some peculiar features of the case are interesting: common manifestations with the hemophagocytic lymphohistiocytosis (HLH)-important hepatosplenomegaly, high triglycerides and ferritin levels, erithrophagocytosis); the autoimmune hemolytic anemia - recently cases of SHML associated with autoimmune lymphoproliferative syndrome (ALPS) have been described. The possibility that SHML represents an acquired disorder of apoptosis has raised a special interest. *Conclusions*. Being an extremely rare disease, SHML was recognized very late, despite its characteristic histopathologic features. The particular severity of the presented case, with extensive involvement, progressive evolution, the life threatening complications (mediastinal syndrome, hemolytic anemia), imposed an intensive treatment.

1113

REACTIVATION OF FETAL HEMOGLOBIN IN BONE MARROW DERIVED CD133+ CELLS HEMATOPOIESIS *IN VITRO*

A. Atashi,[1] A. Hajifathali,[2] S. Kaviani,[1] M. Soleimani[1]

[1]*Tarbiat Modarres University, Iran;* [2]*Shahid Beheshti University of Medical Sc, Timisoara, Iran*

Backgrounds. Switching of fetal hemoglobin (Hb F) in the adults was controlled with mechanisms that are unclear. Understanding of mechanisms underlying this process can be useful for treatment of β globin disorders. *Aims.* We investigated probable synergistic effects of Transforming Growth Factor-β3 (TGF-β3) and Stem cell factor (SCF) on fetal hemoglobin expression in hematopoiesis *in vitro*. *Methods.* The bone marrow was collected from normal person. Mononuclear cells were isolated by density gradient centrifugation and CD133+ cells were isolated using Immunomagnetic beads. Isolated cells have cultured for two weeks in IMDM with 30% FBS supplemented with EPO (Erythropoietin) alone as control group, EPO+SCF (first group), EPO+TGF-β3 (second group) and EPO+SCF+TGF-β3 (third group). Then, RT-PCR and flow cytometry analysis were done for detection of γ globin and Hb F respectively. Also, the Colony assay was accomplished. *Results.* Flow cytometry analysis showed occurrence of 96% Hb F positive cells in differentiated population in presence of EPO, SCF and TGF-β3. This percent was higher than other group. These results were confirmed by increase of γ globin expression detected by semiquantitative RT-PCR in comparison with control. The hematopoietic colony forming assay showed that hematopoietic progenitor cells have ability to forming colony the same as untreated cells. *Summary/Conclusions.* In conclusion, the cytokines or its derivatives that are used in this study can be a suitable candidate for treatment and investigation purposes instead of conventional drugs that can increase the Hb F.

1114

HIGH-DOSE MELPHALAN WITH OR WITHOUT PALIFERMINE IN MULTIPLE MYELOMA : A SELF- CASE-CONTROL STUDY

J. Sonet, C. Doyen, C. Chatelain, S. Pegorer, A. Bosly

Mont-Godinne University Hospital, Yvoir, Belgium

Oral, oesophageal, and lower gastrointestinal tract mucositis is a common complication of high-dose chemotherapy conditioning regimens used with peripheral blood stem-cell transplantations (PBSCT). Severe grades of mucositis are associated with higher morbidity such as infections, need for parenteral nutrition, opioid analgesics and prolonged hospitalization. Moreover, oral mucositis is reported by patients as the worst and most memorable complication of their transplant experience. The KGF (keratinocyte growth factor) palifermin stimulates the growth, differentiation, migration and survival of epithelial cells. Palifermin is now approved in the EU to decrease the incidence, duration and severity of oral mucositis in patients with haematologic malignancies requiring autologous hematologic stem-cell transplantation. Prior to marketing approval, Palifermin was made available to patients in the EU through an early access program. This report describes the treatment of 3 patients who received drug through this program. To evaluate the effect of Palifermin on oral mucositis given during the second autologous transplant in 3 patients who underwent a double PBSCT for multiple myeloma. Three multiple myeloma patients were scheduled to receive treatment with high-dose (HD) Melphalan (200 mg/m^2) followed by two successive autologous PBSCT. At the time of second autograft, patients received prophylactic intervention with intravenous Palifermin 60 µg/kg/day for 3 days before HD Melphalan and for 3 days after PBSCT. Mucositis prevention, hematologic growth factors, parenteral nutrition and all other supportive care were identical during the two PBSCT and followed institutional protocol. Regimen-related toxicity, particularly mucosal toxicity were compared between the first and second PBSCT with each patient representing its own control. Oral mucositis was assessed according to the WHO oral-toxicity scale. 4. Results. Palifermin use during the second PBSCT prevented the occurrence of oral mucositis in all 3 patients, in comparison to the first PBSCT where WHO oral mucositis of grade 3, 2, or 1 in severity was recorded. Pyrosis and abdominal pain lessened in severity in 2 of 3 patients, but the severity of diarrhea did not change (2 patients) or was worse (1 patient). The duration of parenteral nutrition was reduced in 2 patients (15 to 14 days and 13 to 8 days, respectively) and the duration of hospital stay was reduced in 2 patients (21 to 14 days, and 16 to 12 days, respectively) when palifermin was administered with the conditioning regimen. Side-effects of Palifermin were noted in all patients and consisted of pruritis (1), erythema (1), mouth and tongue disorders (3) and edema (1), but were all mild to moderate in severity and self-limiting. 5. Conclusions. In comparison to the first autologous PBSCT after high-dose melphalan where oral mucositis of grade 3, 2 and 1 in severity was recorded without intervention, Palifermin use prevented the occurrence of oral mucositis in all 3 multiple myeloma patients, and lessened the clinical signs of oesophagitis or enteritis in 2 of the 3 patients undergoing double PBSCT. Further evaluation in larger but similar populations would be of interest to confirm these encouraging results.

Table 1. Characteristics of patiets and side effects after double transplantation procedure.

	Patient 1		*Patient 2*		*Patient 3*	
Diagnosis	Myeloma IgG III		Myeloma IgG III		Non secretant Myeloma III	
Interval between PBSCT's	123 days		120 days		109 days	
	PBSC1	*PBSC2*	*PBSC1*	*PBSC2*	*PBSC1*	*PBSC2*
CD 34+ infused	3.2×10^6/kg	3.2×10^6/kg	3.8×10^6/kg	4.3×10^6/kg	3.2×10^6/kg	3.3×10^6/kg
Hospitalization post PBSC	21	14	16	12	15	17
TPN	15	14	10	12	13	8
WHO Grade Oral Mucositis	3	0	2	0	1	0
WHO Grade pyrosis, dyspagia, esophagitis	5	3	2	6	5	2
WHO abdominal pain, colitis	3	1	4	0	0	0
WHO diarrhea	0	3	3	3	1	1

1115

LINEAGE SPECIFIC CHIMERISM ANALYSIS ALLOWS EARLY DETECTION OF RELAPSES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

S. Galeano,[1] R. Gabus,[2] M. Bengochea,[3] J.M. Boiron,[4] E. Carreto,[3] E. Bodega,[2] A.I. Alvarez[3]

[1]*Pro. In. Bio, MONTEVIDEO, Uruguay;* [2]*Hospital Maciel, MONTEVIDEO, Uruguay;* [3]*Inst. Nacional De Donacion Y. Trasplante, MONTEVIDEO, Uruguay;* [4]*Etablissement Francais Du Sang, BORDEAUX, France*

Backgrounds. Chimerism analysis is essential to verify the origin of hematopoiesis after allogeneic stem cell transplantation (SCT). Considering that after SCT, almost all relapses are recipient-derived, the reappearence of mixed chimerism or an increasing fraction of recipient-derived cells should prompt the suspicion of relapse and be differentiated from graft failure or rejection. Furthermore, as reduced intensity conditioning SCT (RIC-SCT) emerge as a frequent procedure, a correct interpretation of chimerism analysis becomes imperative since transient mixed chimerism is frequently observed after RIC-SCT and does not necessarily means an unwanted evolution. *Aims.* To evaluate the usefulness of our methodology of lineage-specific chimerism analysis to sensitively detect relapse early after conventional or RIC SCT. *Methods.* We performed chimerism analysis in whole peripheral blood (PB) as well as in the separated cells on days 14, at the time of neutrophil recovery and monthly thereafter during the first year after SCT. Chimerism was determined on PB by short tandem repeat (STR) analysis on unfractionated PB or after cell separation (lineage-specific chimerism: positive selection of mononuclear cells using CD3, CD15 and CD19 monoclonal antibodies conjugated with magnetic beads; Dynabeads®). DNA was obtained with the Miller method and samples were used in a multiplex polymerase chain reaction to amplify 6 (D8S1130, D21S1270, D6S1031, D3S2406, D9S938, IFNAR-ALU) or 10 (D16S2622, D1S1612, D2S1353, D22S685, D11S1392, D3S2398, D5S2501, D15S657, D10S1237, IFNAR-ALU) STRs loci. Primers were marked with Cy5. Separation and detection of fragments were done with ALF-Express® and infomative peaks were analyzed with the AlleleLinks® software. Depending on the locus, sensitivity to detect mixed chimerism was evaluated in 1 to 5%. *Results.* Fifteen patients were allografted at Maciel Hospital, Montevideo, Uruguay, from january 2003 to december 2004 and those with at least

1 chimerism analysis were included (n=13). Five patients relapsed during the first year after SCT. Three of them were detected by chimerism analysis: in one case, mixed chimerism was observed in the subpopulation compromised by the disease (CD19+ in B lineage ALL with CD19 positive blasts) while in the 2 other patients, relapses were detected by an increasing recipient hematopoiesis in unfractionated blood and CD3-CD19-CD15 subpopulations (AML with CD15+, CD3- and CD19-blasts and ALL with CD19+, CD3- and CD15- blasts). The other 2 patients had relapses of CML that were detected by nested PCR for bcr/abl and cytogenetic analysis but did not show mixed chimerism. Conclusions. These results suggest that, at least in some diseases, lineage specific chimerism could be an alternative to other methods to increase sensitivity and specificity of relapse detection.

1116

ATORVASTATIN INHIBITS THE EXPRESSION OF ADHESION MOLECULES ON ENDOTHELIAL CELLS BY REDUCING REACTIVE OXYGEN SPECIES

I.V. Korzh, V.D. Nemtsova

Kharkov Medical University, KHARKOV, Ukraine

Backgrounds. The migration of circulating monocytes into the subendothelial occurs through the expression of some adhesion molecules on endothelial cells. The nuclear factor (NF)-kappaB, a redox-sensitive element, plays a key role in adhesion molecule gene induction. Aims. Was to investigate the influence of atorvastatin on adhesion molecules expressions. Methods. In this study we have assessed the effects of atorvastatin on the cellular redox state (monitored by measuring intracellular reactive oxygen species and thiol status), expression of adhesion molecules, and activation of NF-κB in human umbilical vein endothelial cells (HUVECs). *Results.* Atorvastatin significantly and dose dependently reduced the intracellular reactive oxygen species (ROS) and superoxide formation induced by oxidized low-density lipoprotein (ox-LDL) (p<0.001) and tumor necrosis factor-α (TNF-α) (p<0.01). Atorvastatin also decreased the consumption of the intracellular GSH induced by ox-LDL (p<0.05) and TNF-α (p<0.01). In addition, atorvastatin significantly and dose dependently reduced the expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (*ICAM-1*), and E-selectin induced by ox-LDL (p<0.01) and TNF-α (p<0.01) on HUVECs. Conclusions. Ox-LDL and TNF-α increased the activation of NF-κB and the preincubation of HUVECs with atorvastatin, dose dependently reduced its activation (p<0.001). Atorvastatin may be useful in inhibiting foam cell formation and thus slow the development of atherosclerosis.

1117

EXPRESSION OF TGFγ ON THE SURFACE OF PERIPHERAL BLOOD CELLS

G. Vartholomatos,[1] S. Paschou,[2] L. Dova,[3] N. Kolaitis,[4] A. Tsatsoulis,[2] G. Papadopoulos[5]

[1]*University Hospital of Ioannina, IOANNINA, Greece;* [2]*Endocrinology Clinic, Ioannina Regional, IOANNINA, Greece;* [3]*Hematology Laboratory-Unit of Molecular, IOANNINA, Greece;* [4]*Ioannina Regional, IOANNINA, Greece ;* [5]*Laboratory of Biochemistry and Biophysic, ARTA, Greece*

Backgrounds. Tissue Growth Factor β is a multifaceted cytokine involved in several important life processes including hemostasis, tissue growth and immune suppression. It is active at pM concentrations and its actions are mediated via cell surface heterotetrameric receptor (TGFγRI-TGFγRII)2 complexes. Recently, several groups have identified TGFγ on the surface of the immunoregulatory $CD4^{+}CD25^{hight}$ (Treg) cells in mouse and man, and certain groups have provided evidence in favor of mediation of the regulatory role by such cells via this cytokine. *Aims.* We have established that the majority of Treg cells in the peripheral blood of normal human donors express TGFβ on their surface, and wished to explore further the possibility that other types of peripheral blood cells might do so as well. *Methods.* Peripheral blood from healthy normal donors (n=16, 5M 11F, aged 20-45) having no first or second degree relatives suffering from autoimmune diseases, was obtained and incubated with an anti-TGFβ-PE antibody, as well as anti-CD4, -CD8, -CD14, -CD15, CD19, and -CD56-FITC labelled antibodies and cells were analysed by flow cytometry. We have also analysed our controls for the presence of Treg cells. *Results.* We have invariably detected TGFβ on the surface of human CD14+ monocytes (24-95% of such cells above the 99.5 percentile of background, with Mean Fluorescence Intensity 124.1, range 37-251, versus MFI of 6.13, range 5.1-7.6, in cells incubated with isotype controls). Of the other peripheral blood cell types, the majority of Tregs in all subjects, showed staining for TGFβ In only a minority of subjects a large fraction of polymorphonuclear cells (MFI 22.1) and a low percentage CD8+ T cells, showed such staining. Comparatively, there is more TGFβ per monocyte than per Treg or polymorphonuclear cell, and the distribution appears more uniform in monocytes and in the PMNs of those subjects expressing the cytokine on their cell surface, than in Tregs *Conclusions.* To our knowledge this is the first report showing expression of this cytokine invariably on monocytes besides Tregs, and we believe that its presence must be considered important for these as yet unexplored biological functions that are under investigation.

1118

EXPRESSION OF CD158B AND CD94:NKG2A ON NK AND CIK CELLS IN MULTIPLE MYELOMA AND NON-HODGKINS LYMPHOMA PATIENTS

M. Biedron,[1] T. Wrobel,[2] G. Mazur,[2] J. Dzietczenia,[2] K. Kuliczkowski[2]

[1]*Wroclaw Medical University, WROCLAW, Poland;* [2]*Dep. of Haematology, WROCLAW, Poland*

Backgrounds. Killer cell immunoglobulin-like receptors (KIRs) and C-type lectin receptors are expressed on natural killer cells (NK) and some T cells, i.e. cytokine-induced killers (CIK, CD56+CD3+ cells). These are MHC class I-specific receptors. Upon binding to MHC class I molecules on target cells, these receptors deliver a negative or positive signal that prevents or activate the NK-mediated lysis of target cells. NK cells and CIK express various KIRs and C-type lectin receptors. There are some reports that expression of these receptors could be changed in neoplasms. *Aim*. The aim of this study was to investigate the expression of KIR (CD158b) and C-type lectin receptor (CD94:NKG2A complex) on NK cells and CIK in multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL) patients. *Methods.* 41 MM (27 Female, 14 Male, mean age: 64 years) and 38 NHL (19 Female, 19 Male, mean age: 59 years) patients were studied. They were studied at diagnosis and after 3 courses of chemotherapy. The control group consisted of 15 age-matched normal donors. The presence of CD158b and CD94:NKG2A was evaluated in NK cells and CIK isolated from patients and normal donors. Peripheral blood mononuclear cells (PBMCs) were obtained by density-gradient centrifugation (Ficoll-Hypaque) of heparinized venous blood.

Table 1.

		NK cells with CD158b [%]	NK cells with CD94 [%]	NK cells CD94+ with NKG2A [%]	NK cells with CD94: NKG2A [%]	CIK with CD158b [%]	CIK with CD94 [%]	CIK CD94+ with NKG2A [%]	CIK with CD94:NKG2A [%]
NHL		30	55	75	42	22	30	62	19
MM		42	49	64	30	26	27	52	14
Control		37	45	87	39	21	19	74	15
P	NHL: Control	NS	NS	NS	NS	NS	NS	NS	NS
	MM: Control	NS	NS	0,003	NS	NS	NS	0,0048	NS
	NHL: MM	0,009	NS	NS	0,009	NS	NS	NS	NS

These were then counted microscopically. Three colour immunofluorescence staining was performed. Seven ml of monoclonal antibodies conjugated with FITC, PE and Cy-Chrome was added to each tube. We used the following monoclonal antibodies specific for: CD14, CD45 (DAKO, Denmark), CD3, CD56, CD94, CD158b and NKG2A (Becton Dickinson, USA). Tubes were than agitated and incubated for 20 min. at 4°C in the dark, after which 5 mL of PBS-$Ca2^{+}Mg2^{+}$ containing 0,1% NaN3 (Sigma Chemical Co., St Louis, Mo, USA) was added to each tube and pelleted by centrifugation (1200 rpm for 10 min). The supernatant was removed and the pellet resuspended in 0,2 ml PBS-$Ca2^{+}Mg2^{+}$ with 0,1% NaN3 paraformaldehyde. 20 000 labelling events were routinely accumulated and analysed for fluorescence on PAS flow cytometer (Partec, Munster, Germany) using FloMax software. The results were statistically analysed using test ANOVA rang Kruskal-Wallis. *Results.* Results are showed in the Table 1. *Conclusion*. we have demonstrated that there are no differences in distribution of CD158b and

CD94:NKG2A in NK and CIK in MM and nHL compared with normal donors. We have showed that the mean percentage of NK and CIK with CD94 expressing NKG2A is lower in MM patients compared with normal donors (p<0,05). It means that there is an increased expression of non-functional CD94 on NK and CIK of myeloma patients.

1119

UNEXPECTED ANTAGONISTIC EFFECT OF RITUXIMAB WITH PROCARBAZINE DISCLOSED DURING AN IN VITRO TESTING OF RITUXIMAB-MEDIATED SENSITIZATION OF B-CELL LINES TO COMMONLY USED ANTICANCER DRUGS

J. Chumchalova,[1] M. Trbusek,[1] S. Bukovska,[1] M. Klabusay[1], Z. Pospisil,[2] I. Vasova,[1] A. Oborilova,[1] D. Dvorakova,[1] J. Mayer[1]

[1]*University Hospital Brno, BRNO, Czech Republic;* [2]*Masaryk University, BRNO, Czech Republic*

Backgrounds. Rituximab is a chimeric monoclonal antibody specific to the CD20-antigene expressed on mature B-lymphocytes. The antibody sensitizes lymphoma cells to differently acting cytotoxic drugs. Although some combinations of cytostatic agents with rituximab have already been tested, there are many others for which no information is available. *Aims.* To analyse some commonly used and some new combinations of rituximab with differently acting cytotoxic agents in vitro using permanently growing B-cell lines. *Methods.* The stable cell lines derived from a follicular lymphoma (WSU-NHL, DHL-4 and DOHH-2) and Burkitt lymphoma (RAMOS) were used for an *in vitro* viability assay. The cell lines were pretreated by 20 μg/mL of rituximab for 72 hours, followed by a subsequent incubation with the cytotoxic drugs (fludarabine, doxobubicin, vincristine, dexamethasone and procarbazine in four different concentrations) for 48 hours. A proliferation activity was estimated using a WST-1 assay. Obtained data were statistically evaluated using multi-way analysis of variance with interactions. The concentrations, presence or absence of pretreatment and plate variability were taken for the fixed effect. A cell cycle after the rituximab pre-treatment was analysed by flow-cytometry with propidium iodide. *Results.* Rituximab significantly decreased an S-phase of the DHL-4 cells, while no prominent effect on cell cycle was observed for the other cell lines. We observed a significantly different sensitivity of follicular lymphoma and Burkitt´s lymphoma cells to vincristine and fludarabine (FL cells were highly sensitive to vincristine and rather poorly to fludarabine, while an opposite effect was seen for BL cells). The rituximab pretreatment sensitized all cell lines to vincristine, while none were sensitized to doxorubicin. Heterogenous results were obtained for the other combinations. A statistically significant influence of the rituximab pretreatment was proved for: dexamethasone at DOHH-2 and RAMOS, fludarabine at WSU-NHL and fludarabine and dexamethasone at DHL-4 cell lines. We obtained quite unexpected results for procarbazine in combination with rituximab. Although the drug strongly inhibited a metabolic activity in all tested cell lines, the effect was just opposite when the cells were pretreated with rituximab. A highly statistically significant antagonistic effect was proved for all the cell lines. *Summary*. The data confirm that rituximab might sensitize lymphoma B-cells to most of differently acting anti-cancer agents. There are, however, some drugs manifesting a strong antagonistic effect with respect to rituximab. Therefore, based on our experimental data, the combination of rituximab with chemotherapy regiments containing procarbazine (e.g. R-COPP) does not seem to be clinically warranted.

1120

ASPIRIN RESISTANCE IN PATIENTS AFTER ISCHAEMIC STROKE AND ISOPROSTANE (8-EPIPROSTAGLANDIN F2A) PLASMA CONCENTRATION

M.Z. Zytkiewicz,[1] E.H. Hanszke,[2] I.A.G. Gaik,[2] Z.T. Turowiecka,[2] L.G. Gielwanowska,[2] P.P. Psuja,[1] K.Z. Zawilska[2]

[1]*ZOZ Poznan-Nowe Miasto, POZNAN, Poland;* [2]*University of Medical Sciencies, POZNAN, Poland*

Background. The limited efficacy of secondary prevention for ischaemic stroke may be partially related to aspirin resistance leading to continuous generation of intraplatelet thromboxane A2. Besides other underlying metabolic mechanisms, an oxidant stress along with nonenzymatic biosynthesis of isoeikosanoids supporting the platelet activation has been suggested. *Aims*. We analyzed the incidence of aspirin resistance in survivors of ischemic stroke and compared the usefulness of some platelet tests designed for its laboratory exploration. *Material and Methods.* Forty four patients, at least a month after acute onset of ischaemic stroke were included into the study. All of them have been receiving 75-150 mg aspirin daily at least for a month. The control group consisted of 12 adequately matched healthy volunteers. The platelet function was investigated by platelet aggregation induced by either ADP (3.5 and 5.0 μM), collagen (2 μg/mL) or arachidonic acid (AA) (0.6 mM) and measurement of closure time on the collagen and epinephrine (Col/Epi) cartridge in PFA-100_ analyzer. Thromboxane A2 metabolite - 11-dehydro Thromboxane B2 (11-dTxB2) and isoeicosanoid '8-epi Prostaglandin F2_ (8-epiPgF2_) plasma concentration by immunoenzymatic method (EIA Kits from Cayman Chemicals) were also determined. The aspirin ingestion was controlled by diminished intraplatelet concentration of malonyldialdehyde. Aspirin resistance has been determined by the following criteria: the intensity of platelet aggregation induced by ADP 60%, collagen 70%, AA 20%, PFA-100_ closure time 165 s and as reference indicator; 11-dTxB2 concentration mean of the control group minus SD.

Table 1. Aspirin resistance in patients after stroke.

The frequency of aspirin resistance in patients after ischemic stroke

		Platelet aggregation			
ADP 3.5 μM	*ADP 5.0 μM*	*Collagen*	*Arachidonic acid*	*PFA-100*	*11-dTxB₂*
45%	52%	20%	7%	52%	43%

Statistically significant inverse correlations have been found between the plasma concentration of 11-dTxB2 and PFA-100_ (Col/Epi) closure time (r=-0.31; p=0.039) as well as between plasma concentration of 8-epiPgF2™ and PFA-100™ (Col/Epi) closure time (r=-0.36; p=0.019). Conclusions. 1. Laboratory tests reveal aspirin resistance in almost half of patients after ischaemic stroke. 2. The most significant correlation has been found between plasma concentration of reference indicator 11-dehydro Thromboxane B2 and PFA-100™ closure time. 3. An important interrelationship observed between PFA-100™ closure time and plasma concentration of 8-epi Prostaglandin F2™ may support the hypothesis of nonenzymatic production of isoprostanoids with platelet proaggregatory activity, playing a role in aspirin resistance.

1121

INDUCTION OF APOPTOSIS IN NB4 CELL LINE TREATED WITH ARSENIC TRIOXIDE AND THE EFFECT OF VIT.D3 ASSESSED BY THE COMET ASSAY

F. Asghari, H. Mozdarani, M. Soleimani

Tarbiat Modares University, TEHRAN, Iran

Backgrounds. Successful treatment of acute promyelocytic leukemia (APL) relies on the ability to kill or arrest the growth of the leukemic blasts. This can be accomplished by inducing maturation, as is the case in differentiation therapy and conventional chemotherapy by induction of Apoptosis. NB4 cells, a model of APL, have shown to undergo monocytic differentiation in response to 1α, 25 dihydroxy Vitamin D3 (1α, 25 D3) and apoptosis or partial differentiation in response to aresnic trioxide (AS_2O_3). A change that usually happens during apoptosis is the severe fragmentation of cellular DNA, a characteristic that can be readily measured by single cell gel electrophoresis, known as the comet assay. Aims. Study of the effects of arsenic trioxide (ATO) and Vit.D3 on induction of apoptosis in NB4 cell line using the neutral comet assay. *Methods.* NB4 cells were treated with various doses of arsenic trioxide (0.1-3 μmol) and Vit.D3 (100-600 nM) alone or combined together. 24 hours later cells were mixed with low melting point agarose and placed onto a precoated slide. After lysis and electrophoresis in neutral condition, cells were stained with ethydium bromide and observed under a fluorescent microscope. The data were then analyzed and compared. Results. Results show that ATO induced apoptosis in NB4 cells at all doses used in this study. The effect was dose and time dependent and significantly different from controls (p<0.05). In contrast, Vit.D3 at concentrations of 100-600 nM showed no effect on induction of Apoptosis. Treatment of the NB4 cells with arsenic trioxide in combination with Vit.D3, a monocytic inducer, resulted in reduction of apoptosis as compared to arsenic trioxide alone at the same concentration (p<0.05) in all groups. Conclusion: Results show clearly that ATO is a potent inducer of apoptosis in NB4 cells and the effect is dose and time dependent. On the other hand, the results suggest that Vit.D3 decreases the sensitivity of cells to arsenic trioxide. A significant decrease in apoptosis in the various treatment groups, clearly gives evidence that Vit.D3 has a protective role (in this combination). Also neutral comet assay can be considered as a suitable method for detection of chemically induced apoptosis

1122

A NOVEL T(4;17)(Q12;Q21) WITH REARRANGEMENT OF THE 17Q21 RARA LOCUS IN A CASE WITH JUVENILE MYELOMONOCYTIC LEUKEMIA

A. Buijs, M.C.A. Bruin

UMC Utrecht, UTRECHT, Netherlands

Translocations involving the *RARA* locus on 17q21 have been identified in acute promyelocytic leukemia (APL). The majority of APL harbors the t(15;17)(q22;q21), resulting in a *PML-RARA* fusion transcript. Variant rearrangements involving RARA in APL are t(11;17)(q23;q21)(PLZF-RARA), t(5;17)(q35;q21)(NPM-RARA), t(11;17)(q13;q21)(NUMA-RARA), a der(17)(STAT5b-RARA) and t(3;17) (p25;q21). Here we report a case of juvenile myelomonocytic leukemia (JMML) carrying a t(4;17)(q12;q21) with a rearrangement of the RARA locus at 17q21 demonstrated by FISH (probe LSI RARA DCBA, Abbott-Vysis). FISH analysis using BAC probes derived from the 4q12 region indicated that the breakpoint is located proximal of the CHIC and PDGFRA loci. The more proximally located FIP1L1 gene at 4q12 remains an attractive candidate gene. We will present ongoing FISH studies using probes spanning the 4q12 FIL1P1 locus and experiments to test whether the translocation results in FIL1P1-RARA fusion gene. This is the first report on a rearrangement of the RARA locus at 17q21 in JMML until now exclusively demonstrated in APL.

1123

NUP98/HOMEOBOX HEMATOPOIETICALLY EXPRESSED (HHEX) FUSION GENE IN ACUTE MYELOID LEUKEMIA WITH T(10;11)(Q23.3;P15.5)

R. La Starza,[1] P. Gorello,[1] R. La Starza,[1] R. Rosati,[1] A. Santoro,[2] V. Rizzo,[2] V. Pierini,[1] M. Negrini,[3] M.F. Martelli,[1] J. Schwaller[1], C. Mecucci[1]

[1]*Hematology, University of Perugia, PERUGIA, Italy;* [2]*Hematology, V Cervello Hospital, PALERMO, Italy;* [3]*Center for Cancer Research, FERRARA, Italy*

Background. Chromosomal traslocations involving the 5' region of the nucleoporin gene, NUP98, on chromosome 11p15.5 emerged as recurrent leukemogenic events in myeloid and T lymphoid malignancies. Among the twenty partners identified sofar there are both homeobox and non-homeobox, coiled-coil containing proteins. *Aim*. Identification of NUP98 recombinations in hematological malignancies with 11p15 abnormalities and characterization of new partners. *Methods*. We performed cytogenetic and molecular studies in a 59-year-old man at second relapse of acute myeloid leukemia. Bone marrow karyotype was 46,XY,t(10;11)(q23;p15) in 15/15 metaphases. To study NUP98 involvement, metaphase FISH was done with the DNA clone RP5-1173K1 spanning exons 10 to 20 of NUP98. 3' RACE'PCR experiments were performed using NUP_1083_F (5'-ggtaataccagcaccataggacag-3') as a gene-specific primer. RT-PCR was performed with gene-specific primers NUP 1284_F (5'-cttactacatttggaagcag-3') and HHEX_606_R (5'-atttagcgcgtcgattctga-3') to confirm the NUP98/HHEX chimeric transcript. The presence of HHEX/NUP98 chimeric transcript was investigated with HHEX_346_F (5'-ggacggtgaacgactacacg-3') and NUP98_1861_R (5'-agccatcaaagagatgtg-3'). The PCR products were subcloned in pGEM-T easy vector and sequenced. *Results*. RP5-1173K1 gave three hybridization signals on normal 11, on der(11) and on der(10) indicating NUP98 was disrupted by traslocation. 3'-RACE-PCR identified a fusion between *NUP98* and *HHEX* which was confirmed by RT-PCR. Three different in-frame spliced transcripts between NUP98 exon 13 and HHEX exon 2 were detected. Isoforms differed for the nucleotide number in the 5'portion of NUP98, upstream of the breakpoint region. An out-of-frame reciprocal *HHEX* exon 1/ NUP98 exon 14 fusion was also found. Based on molecular findings clone RP11-469M1 was selected for the *HHEX/10q23.3* gene and a double colour double fusion FISH assay was set up to document the *NUP98/HHEX* fusion in metaphase and interphase nuclei. *Conclusion*. The *HHEX* gene encodes a member of the homeobox family of transcription factors. It is the 10th homeobox gene involved in NUP98 rearrangement and the second NUP98 partner located on the long arm of chromosome 10. Our double colour fusion FISH assay is helpful to discriminate between t(10;11)(q25;p15)/NUP98-ADD3 and t(10;11)(q23;p15)/NUP98-HHEX. Identification and characterization of NUP98 translocation partners is an important step to unravel the leukemogenic mechanism(s) underlying NUP98 recombinations.

Funding. Supported by MIUR, FIRB and Fondazione Cassa di Risparmio di Perugia.

Acknowledgements. We wish to thank Dr M Rocchi (University of Bari, Italy) for providing DNA RP11 and RP5 clones.

1124

PROGNOSTIC SIGNIFICANCE OF CYTOGENETIC AND MOLECULAR CHANGES IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

O. Haus,[1] K. Soszynska,[2] B. Mucha,[2] E. Duszenko,[3] K. Skonieczka,[2] R. Debski,[4] M. Wysocki[4]

[1]*Dept. of Clinical Genetics, CM UMK, BYDGOSZCZ, Poland;* [2]*Dept.of Clinical Genetics, CM UMK, BYDGOSZCZ, Poland;* [3]*Dept. of Hematology, Medical University, WROCLAW, Poland;* [4]*Dept. of Pediatrics CM UMK, BYDGOSZCZ, Poland*

Chromosome aberrations and molecular rearrangements are closely associated with a particular morphologic or immunophenotypic subtype of childhood acute lymphoblastic leukemia (ALL). An integral part of diagnostic process in ALL patients is cytogenetic and molecular analysis. The aim of the study was to assess a prognostic value of genetic changes in childhood ALL. The total number of 70 children with newly diagnosed, untreated ALL, ranged from 1 month to 18 years of age, were included in the study. Conventional cytogenetics and RT-PCR analyses of fusion genes: *BCR/ABL*, *MLL/AF4*, *E2A/PBX1* and *TEL/AML1* were performed in all patients. Metaphase and interphase FISH technique with dual color translocation probes was used to verify the presence of fusion genes and to estimate the percentage of cells bearing them. To detect the rearrangements of MLL gene a split signal probe was used. Statistical analyses were performed with the help of Statistica program with Kaplan-Meier method and Cox multiple proportional hazard model. Chromosome preparations were obtained in 59 of 70 (84%) cases. 35 of 59 (59%) cases revealed chromosome aberrations. RT-PCR results were obtained in all 70 cases. Hyperdiploidy >50 chromosomes was present in 9 cases; in 6 cases only numerical (trisomies) and in 3 both numerical and structural aberrations were found. A hyperdiploidy 47-50 chromosomes was present in 6 patients, pseudodiploidy in 15 and hypodiploidy in 5. The fusion gene BCR/ABL was present in 2 out of 70 patients, PBX1/E2A in 2, and TEL/AML1 in 14. MLL/AF4 was not found but FISH with MLL split probe revealed the rearrangements of MLL (11q23) in 4 patients. The probability of event-free survival time (pEFS) and of total survival time (pST) was the highest in the groups of children with hyperdiploidy >50 chromosomes or with TEL/AML1 fusion, both without other changes, structural or numerical. Moreover, TEL/AML1 was a good prognostic factor only when present in a high percentage (>80%) of examined cells. The presence of pseudodiploidy or hypodiploidy correlated in general with moderate or poor outcome. The outcome for a group of patients with one of the following: t(1;19), t(9;22) or 11q23 rearangement, was the worst and pEFS significantly lower than in the remaining patients ($p<0.05$). The most unfavorable independent risk factors were MLL rearrangements and BCR/ABL. The presence of MLL rearrangements caused 12-times increased, and BCR/ABL - 8-times increased risk of relapse or treatment failure. The WBC and early response to induction therapy were significant ($p<0.05$), independent hematological and clinical risk factors in ALL patients. The results of the study confirm the prognostic value of cytogenetic, FISH and molecular analyses in childhood ALL and underline the need of using them together at the diagnosis of ALL to establish the prognosis of the disease.

1125

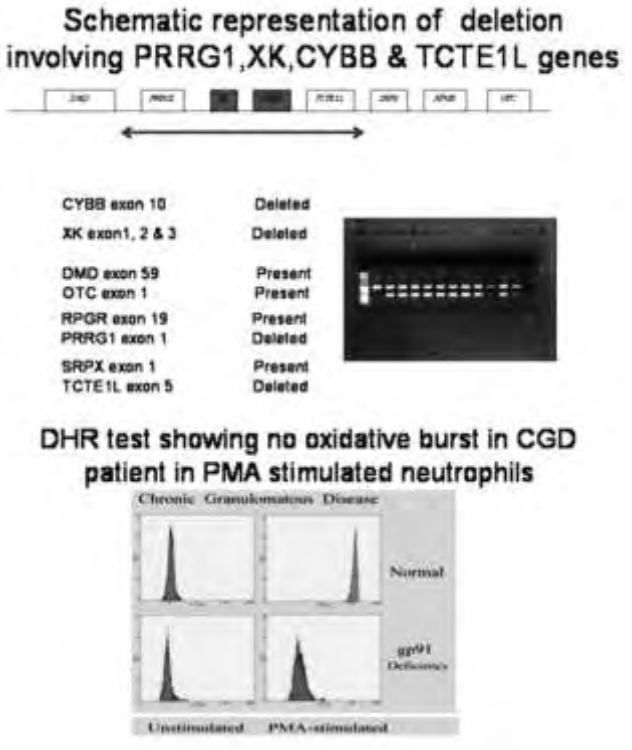
MOLECULAR ANALYSIS OF X LINKED CHRONIC GRANULOMATOUS DISEASE & MCLEOD SYNDROME : A CASE REPORT

S. Al Zadjali,[1] I. Elnour,[1] A.V. Pathare,[1] S. Al Kindi[1], A. Al Mammari,[1] S. Al Mammari,[1] B. Al Said,[1] D. Dennison[1], R. Krishnamoorthy[2]

[1]*Sultan Qaboos University, MUSCAT, Oman;* [2]*Hopital Robert Debre, PARIS, France*

Backgrounds. Chronic granulomatous disease [CGD] is a rare inherited disorder of the immune system, characterized by severe susceptibility to infection, due to failure of phagocytic leukocytes to generate microbicidal reactive oxygen species. Defects in any of the four components of the *NADPH* oxidase, [p47phox, p67phox,p22phox or gp91phox] a multicomponent enzyme complex, encoded by *NCF1*, *NCF2*, *CYBA* and *CYBB* genes respectively can give rise to CGD, the most common being the X-linked form of CGD due to mutations located the *CYBB* gene. *Aims.* To characterize the molecular lesion in an Omani patient with X-Linked Chronic Granulomatous Disease. *Methods.* Clinical suspicion of CGD was confirmed with dihydrorhodamine reduction [DHR test] by PMA-stimulated neutrophils as an initial diag-

nostic test by Flowcytometry. Genomic DNA was isolated from peripheral blood leukocytes of the affected patient and two siblings and his father by Qiagen DNA extraction Kit. Analysis for the presence of specific genomic coding sequences in several genes at the Xp21 locus namely DMD exon 59; PRRG1 exon 1; XK exons 1,2,3; CYBB exon 10; TCTE1L exon 5; SRPX exon 1; RPGR exon 19 and OTC exon 1 were amplified by PCR reaction using specific primers. The presence of expected PCR products were reconfirmed by and documented by gel electrophoresis using an internal control gene. Additional studies were also performed to evaluate the Kell antigen system on the red blood cells. *Results.* DHR test showed no oxidative burst consistent with the diagnosis of CGD.



It was observed that the patient had a large deletion extending from PRRG1 to TCTE1L genes (Figure) with loss of both XK and CYBB genes. Flow cytometry showed weak expression of Kell antigens on the red blood cells of the patient. *Summary/Conclusions.* This study illustrates the rare event in our patient presenting with clinical manifestations of CGD and McLeod's syndrome owing the underlying deletion of *XK*, *CYBB*, *PRRG1* and *TCTE1L* genes.

1126

IDENTIFICATION OF THE V617F JAK2 MUTATION IN MYELOPROLIFERATIVE DISORDERS

N. Salgueiro,[1] I. Salgado,[1] R. Duarte,[2] R. Bauerle,[2] I. Castro,[3] A. Espirito Santo,[3] A. Carneiro,[3] L. Francisco,[4] A. Macedo,[4] M. Gomes,[5] S. Castedo[1]

[1]*GDPN, PORTO, Portugal;* [2]*Centro Hospitalar do Alto Minho, VIANA DO CASTELO, Portugal;* [3]*Hospital de S. Joo, PORTO, Portugal;* [4]*Centro Hospitalar da Cova da Beira, COVILHA, Portugal;* [5]*IPO-CROC, COIMBRA, Portugal*

Backgrounds. Polycythemia Vera (PV), Essential Thrombocythemia (ET), Myelofibrosis (MF) and Chronic Myelogenous Leukemia (CML) were grouped into a spectrum of related disorders by Dameshek in 1951, dubbed Chronic Myeloproliferative Diseases (MPDs). However, a disease-causing genetic alteration (BCR/ABL rearrangement) has been identified only in CML. The other 3 disorders (ET, PV and MF) are classified as the BCR/ABL negative classics MPDs and are therefore distinguished from CML. The discovery of a single mutation in the Janus Kinase (JAK)-2 gene (substitution of a valine for a phenylalanine in the codon 617) in a high percentage of cases of PV, ET, MF suggests that it may be the underlying molecular mechanism for these disorders. This single mutation has been reported in 65-97% of patients with PV, 23-57% of ET cases, 35-57% of MF cases and in 20% of patients with unclassified MPDs. The identification of JAK2 mutation represents a major advance in our understanding of the molecular pathogenesis of MPDs and provides a hallmark of genetic alteration in these disorders. Aims and Methods. We studied 57 Portuguese patients with MPD: 32 patients with MPD-NOS (not otherwise specified), 11 with PV, 11 with ET and 3 with idiopathic MF. In each case, DNA obtained from bone marrow or peripheral blood cells was amplified by PCR using specific primers for exon 12 of the JAK2 gene. The mutation V617F was detected by RFLP. *Results.* Analysis of 32 MPDs-NOS revealed in 11 (46,9%) the V617F mutation. This mutation was also identified in 72.7% cases of PV and 27.3% of ET cases. V617F mutation was not identified in the 3 cases with idiopathic MF. *Conclusions.* The results of our study are in keeping with published reports, showing that V617F mutations are very frequent in MPDs, mainly in PV and in ET. These data suggest that the V617F mutations participate in the pathogenesis of chronic MPDs and will probably lead to a new classification of these diseases, contribute to a better stratification of patients according to prognosis, and hopefully allow the development of novel therapeutic approaches.

1127

MOLECULAR DIAGNOSIS OF β- THALASSAEMIA IN ROMANIA: THE FIRST APPLICATION TO PRENATAL DIAGNOSIS

D.C. Coriu,[1] R.T. Talmaci,[2] L.D. Dan,[3] S.K. Klio,[4] L.B. Barbarii,[5] R.V. Vladareanu,[6] M.D. Dogaru,[1] D.C. Colita[1]

[1]*Fundeni Hospital, BUCHAREST, Romania;* [2]*Genetics Institute of Bucharest Universi, BUCHAREST, Romania;* [3]*Genetics Institute, BUCHAREST, Romania;* [4]*National Thalassaemia Center, Laikon Gen, ATHENS, Greece;* [5]*National Forensic Institute of Legal Med, BUCHAREST, Romania;* [6]*Elias Emergency Universitary Hospital, BUCHAREST, Romania*

Thalassemia major is a classical example of a disease that can be prevented by prenatal diagnosis. In Romania there are currently 300 patients with thalassemia major under the management of specialized institutions. So far, the prenatal diagnosis of thalassaemia was not available in Romania, for various reasons. For the prenatal diagnosis of β-thalassemia the first step is the characterization of the spectrum of mutations causing this disease in the Romanian population. In 2003 our institution, benefiting from the help of the Romanian Academy of Science initiated a Screening Programme for thalassaemia having as a main purpose to perform a screening for β-thalassaemia mutations previously described in the Romanian population. *METHOD*: Haematological data were collected with automated cell counters (Coulter). Quantification of haemoglobin was done by cation exchange HPLC and by agarose gel electrophoresis. Analysis of the mutation in the β-globin gene has been performed by using the PCR based Methods. Amplification Refractory Mutation System (ARMS), restriction enzyme analysis and Denaturing Gradient Gel Electrophoresis (DGGE). Results. Until now we have identified 11 β'thalassaemia alleles: IVS I-110 (37,88%), CD 39 (13,64%), IVS II-745 (13,64%), IVS I-6 (13,64%), IVS I-1 (7,58%), -87 (3,03%), CD 5 (3,03%), CD 6 (3,03%), CD51 (1,51%), +22 (1,51%), polyA (1,51%). Using this experience we were able to perform the first prenatal diagnosis for a young couple at risk for thalassaemia major: maternal genotype IVS I'110 / Normal and paternal genotype IVS II'745 / Normal. Fetal samplings were collected by amniocentesis in the second trimester. Maternal contamination of the fetal DNA was ruled out by STR genotyping. Fetal genotype was IVS I-110 / IVS II'745 compatible with the presence of β-thalassemia major. These results were confirmed by the DNA analysis performed in National Thalassaemia Center from Athens, Greece. *CONCLUSION*: The results of this study point to a successful future prenatal diagnosis of β-thalassaemia in Romania, using a rapid and accurate molecular method. Together with the implementation of proper preventive health measures and the education of the parents regarding their carrier status, we are hoping that this method will be used as the common application approach to decrease the incidence of thalassemia major.

Funding. This work was supported by grant 48 and 49/2005 CEEX.

1128

DEVELOPMENT OF A QUANTITATIVE METHOD FOR ASSESSMENT OF BCR-ABL TRANSCRIPTS BY TAQMAN TECHNOLOGY FOR THE LIGHTCYCLER INSTRUMENT

G.M. Garofalaki, I. Baltathakis, I. Tziotziou, E. Nikolou, C. Besleme, D. Karakasis, N. Harhalakis, E. Nikiforakis

Evaggelismos hospital, ATHENS, Greece

The quantification of BCR-ABL transcripts is mandatory for the fol-

low-up of patients with chronic myeloid leukemia who are treated with imatinib or allogeneic hematopoietic stem cell transplantation. Quantification is currently accomplished by means of various fluorescence-based detection techniques which allow for the monitoring of the amplification process in the so called real-time PCR. The recent availability of instruments for real-time quantitative PCR (RQ-PCR) has prompted the development of quantitative methods for the most common fusion transcripts detectable in hematologic malignancies. However, because the ABI PRISM apparatus (Applied Biosystems) was the first available device for real-time PCR, most of the assays were developed with the use of the TaqMan probe chemistry. Upon introduction of other real-time PCR instruments, such as the LightCycler (LC; Roche), different methodologies were described. The aim of this study was to optimize the protocol established for ABI PRISM by J. Gabert *et al*. under the Europe Against Cancer Program (*EAC Protocol, April 2002*) for use with the LC. In addition, the aim was to establish a standard approach of fluorescence data acquisition. The reverse transcription protocol was applied with the use of Superscript reverse transcriptase, starting from a total RNA amount extracted from at least 5×10^6 cells. The PCR reaction mix for the BCR-ABL was prepared in a final volume of 20 μL containing 300ng of each primer, 200 nM of the BCR-ABL TaqManTM probe, 2 μL of FastStart DNA Master Hybridization Probes (Roche), 3 mM MgCl2, 1 unit Uracil-DNA glygosylase and 5 μL of cDNA from patient samples or plasmid DNA dilutions for the creation of the standard curve. The PCR reaction mix for the ABL contained 300ng of each primer and 200nM of the ABL TaqManTM probe. The LC PCR program consisted of an initial denaturation in 95oC for 12 min, followed by 45 cycles of 95oC for 10 sec, and 60oC for 60 sec with fluorescence reading at F1 channel. Data analysis was performed in the F1/F2 mode and quantification was obtained by selection of the *fit points* option. We adjusted the noise band at 0.02 to eliminate background signals. For the analysis step, the crossing line was set at 0.1. In 47 consecutive experiments, the median ± SD of the slope of the calibration curve for BCR-ABL and ABL was -3.555±0.16 and -3.574±0.29 respectively. The median±SD of the PCR efficiency for BCR-ABL and ABL was 1.91±0.06 and 1.9±0.11 respectively. The median ± SD of the intercept for BCR-ABL and ABL was 40.09±1.19 and 41.48 ± 1.6 respectively. Consequently, the values of all the above parameters, that determine PCR efficiency, were within the acceptable limits as defined by the EAC Protocol. The variation in Cp values was less than 1.5 (when Cp value <36) indicating the reproducibility of the RQ-PCR analysis. In conclusion, the TaqMan technology-based protocol can be successfully applied to the LC device with the proposed fluorescence data acquisition method, which provides accurate quantification of BCR-ABL transcripts in accordance with the guidelines of the EAC Protocol.

1129

TRANSCRIPTIONAL PROFILING OF EPSTEIN-BARR VIRUS (EBV) GENES AND HOST CELLULAR GENES IN NASAL NK/T-CELL LYMPHOMA AND CHRONIC ACTIVE EBV INFECTION

Y. Zhang,[1] J. Ohyashiki,[1] R. Hamamura,[1] T. Takaku,[1] N. Shimuzu,[2] K. Ohyashiki[1]

[1]*Tokyo Medical University, TOKYO, Japan;* [2]*Tokyo Medical and Dental University, TOKYO, Japan*

Backgrounds. Nasal NK/T-cell lymphomais an aggressive subtype of non-Hodgkin lymphoma (NHL) that is closely associated with Epstein-Barr virus (EBV). The clonal expansion of EBV-infected NK or T cells is also seen in patients with chronic active EBV (CAEBV) infection, suggesting that two diseases might share a partially similar mechanism by which EBV affects host cellular gene expression. *Aim.* To understand the pathogenesis of EBV-associated NK/T-cell lymphoproliferative disorders (LPD) and design new therapies. Methods. We employed a novel EBV DNA microarray (HHV-4 Viruchip) to compare patterns of EBV expression in six cell lines established from EBV-associated NK/T-cell LPD. We also analyzed the gene expression patterns of host cellular genes using an Affymetrix U133plus2.0 chipset. Results. We found that expression of BZLF1, which encodes the immediate'early gene product Zta, was expressed in SNK/T cells. We identified a subset of pathogenically and clinically relevant host cellular genes which might be a putative contributor for tumor progression. CONCLUSION: This study describes a novel approach from the aspects of viral and host gene expression which could identify novel therapeutic targets in EBV-associated NK/T-cell LPD.

1130

GENE EXPRESSION PROFILING AND DETERMINATION OF GENETIC HETEROGENEITIES AS PROGNOSTIC FACTORS IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH CONVENTIONAL VERSUS NOVEL AGENTS IN CORRELATION WITH OUTCOME

A. Broyl,[1] E. Kamst,[1] D. Hose,[2] Y. de Knegt,[1] H.M. Lokhorst,[3] H. Goldschmidt,[2] P. Sonneveld[1]

[1]*Erasmusmc, ROTTERDAM, Netherlands,* [2]*University of Heidelberg, HEIDELBERG, Germany,* [3]*University Medical Center Utrecht, UTRECHT, Netherlands*

The standard treatment of newly diagnosed multiple myeloma (MM) patients is based on induction treatment followed by high-dose melphalan. CR/nCR percentages range from 20-50% with event free survival (EFS) ranging from 18 months to 28 months. The 5-year survival rates are 25% to 50%, however all patients eventually relapse and succumb to the disease. In patients with unfavourable prognostic factors such as a high serum β2-microglobulin and/or a deletion of chromosome 13, or in elderly patients the prognosis remains poor. Recently, several promising new agents were developed, which interfere with critical cell-survival pathways in myeloma. Amongst these agents, Bortezomib, a proteasome inhibitor, and Thalidomide, an anti-angiogenic and immunomodulatory drug, have a remarkable effect in patients with relapsed or refractory MM with 30-40% response rates. In combination with Dexamethasone and/ or other conventional agents overall response rates of 50-70% can be achieved. In newly diagnosed patients the response rates vary from 70 - 85%. Moreover, Bortezomib was found to overcome poor prognostic factors like a high β2-microglobulin and/ or deletion of chromosome 13. However, 15-30% of newly diagnosed patients do not respond to Bortezomib or Thalidomide. Secondly, 30% of the patients treated with these novel agents have to stop prematurely because of intolerable side effects, such as polyneuropathy, thrombocytopenia, thrombosis and gastro-intestinal symptoms. To gain new insights into the mechanisms of drug response and toxicity associated with these agents, we have embarked on a prospective study to analyze gene expression profiles of myeloma specific genes in plasma cells purified from bone marrow from myeloma patients at diagnosis who have been treated with these novel agents in order to learn which genes govern the response, PFS and OS upon treatment with Bortezomib and Thalidomide. Gene expression profiling of CD138 magnetic cell selected (MACS) myeloma plasma cells will be performed using Affymetrix GeneChip Human Genome U133 plus 2.0 arrays. In addition, we will perform a Single Nucleotide Polymorphism analysis of germline DNA to identify gene mutations that are involved in drug metabolism and treatment-related toxicity. This program has been initiated in a large multicenter, prospective, randomized phase III trial, comparing Bortezomib in combination with Adriamycin, Dexamethasone (PAD, arm A) followed by high dose therapy with stem cell rescue and maintenance therapy with Bortezomib vs. Vincristine, Adriamycin and Dexamethasone (VAD, arm B) followed by high dose therapy with stem cell rescue and maintenance therapy with Thalidomide (HOVON65/GMMG-HD4). This cooperative trial in the Netherlands and Germany has started in April 2005 and will include 800 patients. Data obtained from SNP-array and micro-array studies will be submitted to Cox regression analysis and multifactorial analysis with the clinical data set from these patients. We will present the initial results including an unsupervised cluster analysis based on the array results from the first cohort of 50 - 75 patients.

1131

AUGMENTATION OF TAXAN-INDUCED CYTOTOXICITY OF HL 60 MYELOID LEUKEMIA CELLS BY SERINE/THREONINE PROTEIN PHOSPHATASE INHIBITORS

F. Sahin,[1] G. Saydam,[1] H. Aydin,[1] N. Selvi,[1] G. Oktem,[1] K.P. Ozen,[1] S.B. Omay[2]

[1]*Ege University Hospital, BORNOVA IZMIR, Turkey;* [2]*Karadeniz Teknik niversitesi Tip Fak., TRABZON, Turkey*

Backgrounds and aim. Paclitaxel and docetaxel (taxans) are the agents which ameliorate the function of microtubules in cancer cells, and they have been in clinical use for a long time especially in the treatment of solid tumors. There have been several studies investigating the potential role of these drugs in the treatment of myeloid malignancies. We have previously demonstrated the relationship between serine/threonine protein phosphatase system and taxan induced cytotoxicity of breast cancer cells. It was demonstrated in this study that protein phosphatase inhibitors increased the cytotoxic effect of taxans in MCF7

breast cancer cell line. The aim of our study is to investigate the potential role of serine/threonine protein phosphatase system and specific inhibitors of this system in docetaxel/paclitaxel induced cytotoxicity on HL 60 cells. *Materials and method*. HL60 myeloid leukemia cell line was used as the model cell line. IC50 dose of paclitaxel and docetaxel were found as 20 nM and 5 nM respectively by using trypan blue dye exclusion and XTT assays. Specific inhibitors of protein phosphatase 1 and 2A, okadaic acid and calyculin A were used for further combination studies with the IC50 dose of 5 nM and 0.5 nM, respectively. Acridine orange/ethidium bromide and Hoechst 33342-PI methods were used for evaluation of taxan-induced apoptosis of HL60 cells. Western blotting with specific antibodies against protein phosphatases was used to determine the changes in the expression of protein phosphatases after incubation of cells with taxans. Protein phosphatase activities were assessed by using specific ELISA kits. *Results.* Treating Hl 60 cells with docetaxel and paclitaxel resulted in dose and time dependent cytotoxicity with 24 hours intervals. Combination studies of these drugs with phosphatase inhibitors showed significant increase in the taxan-induced cytotoxicity of HL 60 cells. Acridine orange/ethidium bromide and Hoechst 33342-PI methods confirmed the taxan-induced apoptosis of leukemic cells. Protein phosphatase 1 and 2A activity was found to be increased after treating cells with docetaxel and paclitaxel at maximum level of 72th hour. Western blotting results showed the increase in the expression of protein phosphatase 2A catalytic subunit at 72th hours of incubation. *Conclusion*: Serine/threonine protein phosphatase system has significant role in taxan-induced cytotoxicity against leukemic cells. Potential use specific protein phosphatase inhibitors in combination with taxans will open new windows in the treatment of myeloid leukemias.

1132

A NEW FORMULA FOR DIFFRENTIATION OF IRON DEFICIENCY ANEMIA (IDA) AND THALASSEMIA TRAIT (TT)

M.A. Ehsani,[1] A. Darvish,[2] A. Aslani,[3] F. Seighali[4]

[1]*Tehran University of Medical Sciences, TEHRAN, Iran;* [2]*Atyehsazan e Hafez Company, TEHRAN, Iran;* [3]*Tehran University of Medical sciences, TEHRAN, Iran;:* [4]*AJA University of Medical Sciences, TEHRAN, Iran*

Introduction: The most commonly encountered disorders with mild microcytic anemia are iron deficiency anemia and thalassemia trait. Sensitivity and specificity of many discrimination indices have been reported using red blood cell indices. Youden's index provides an appropriate measure of validity of a particular technique or question by taking into account both sensitivity and specificity. We compare the Youden's index for these indices as well. Methods. We studied 284 individuals with microcytic anemia aged between 6 month and 75 years. There were 188 females and 96 males involved in our study with mean age equal to 24.23(SD, 15.44). Ferritin, HbA2, and Complete Blood Cell (CBC), in which RBC, hemoglubolin (Hb), Hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH), and Mean Corpuscular hemoglobin concentration(MCHC), were measured for all the participants. We diagnosed individuals with HbA2>3.4% as patients with β thalassemia (*BTT*) and those who has a serum ferritin <12ng/mL or respond to administered Iron and anemic situation in their blood subsides as patient suffering from Iron deficiency anemia (IDA). England Index, Mentzer Index, Srivastava Index, Kawakami Index, have been calculated for all formulas as well as OUR INDEX (Ehsani Index) = MCV-(10*RBC) Blood Counts were obtained by H1 Technicon Cell Counter System while ferritin was measured and HbA2 value determined by electrophoresis. Sensitivity, specificity, Positive IDA Predictive Value (PPV), Negative IDA (BTT) Predictive Value (NPV), and Youden Index (YI) was calculated. *Results.* Considering the above criteria we diagnosed 130 patients with BTT and 154 patients with IDA. Sensitivity and specificity for England Index was 99.2 and 69.5, Mentzer Index 94.6 and 95.5, Srivastava Index 88.5 and 85.7, Kawakami Index 86.2 and 98.1, and Ehsani's Index 90.0 and 95.5. *Conclusions.* The most frequently encountered diseases with microcytic anemia are TT and IDA. Screening for TT is of great importance in order to address the patient to a genetic counselor. Iron should not be administered to patients with TT as an attempt to normalize MCV so differentiating TT from IDA has a great importance. Decreased levels of SI, TS and ferritin with increased levels of SIBC are the main diagnostic criteria for IDA. The diagnosis of BTT is established by the presence of characteristic red blood cell microcytosis and elevated levels of HBA2. However in some mutations of BTT and in heterozygous α thalassemia, HBA2 is not elevated. The use of Ehsani Index is so easy and anybody can subtract the ten fold RBC from MCV in mind with no need for calculator.

1133

α THALASSEMIA IN BAHRAIN

K. Shome,[1] S. Abuamer,[2] A. Al Ajmi,[1] N. Jassim,[1] J. Saleh Ali,[3] G. Ameen,[3] K. Sharif,[1] N. Mahdi,[1] S. Al Arrayed,[1] A.A. Satir[2]

[1]*Salmaniya Medical Complex, MANAMA, Bahrain;* [2]*Pathology, CMMS, Arabian Gulf University, MANAMA, Bahrain;* [3]*Bahrain Defence Forces Hospital, WEST RIFFA, Bahrain*

α-thalassemia is one of the commonest genetic disorders in the Arabian Gulf region and its reported incidence varies from 15% to 50% in the different countries in this region. Despite this widespread occurrence there are few comprehensive studies that describe genotype-phenotype correlations in this disorder as observed in this geographic location. To correlate the genotypes of α-thalassemia in Bahraini subjects with the respective phenotypic characteristics. Forty α-thalassemia cases were selected from patients referred by participating hematologists for the investigation of anemia or unexplained microcytosis. The following tests were done for each patient: (i) measurement of hemoglobin and red cell indices (ii) staining for HbH inclusions (iii) analysis of hemoglobin and quantitation of hemoglobin fractions by HPLC and (iv) molecular genotyping using a PCR-based strategy to identify the four common α-thalassemia haplotypes prevalent in the region ($-\alpha^{3.7}$, $-\alpha^{4.2}$, αHphα and αTSaudiα). The assessment of clinical severity was based on the degree of anemia, the requirement for and number of episodes of transfusion and age at first transfusion.The αTSaudiα/haplotype was the most common with a frequency of 41.9% among all haplotypes. This was followed successively by $-\alpha^{3.7}$/(37.8%), αHphα/(10.8%) and $-\alpha^{4.2}$/(9.5%). The homozygous αTSaudi genotype was characterized by presence of high numbers of cells containing intraerythrocytic inclusions of HbH with typical morphology, markedly altered erythrocyte indices especially the RDW, high levels of dyshemoglobin (Hb Bart's and/or HbH) and greater clinical severity. The other genotypes showed overlapping phenotypic features but none were severely affected. The homozygous αTSaudi abnormality is the only genotype in Bahrain with a distinctive phenotype that is identifiable by routine laboratory tests and accounts for almost all the severely affected cases. Premarital screening programs in the region should take these considerations into account when screening strategies are formulated.

1134

EFFICACY OF HYDROXYUREA (HU) IN REDUCTION OF PACK RED CELL TRANSFUSION REQUIREMENT AMONG CHILDREN HAVING β-THALASSAEMIA MAJOR: KARACHI HU TRIAL (KHUT)

S. Ansari, S. Shamsi, S. Shamsi, T. Farzana, M. Muhammad, M. Muhammad, K. Panjwani, K. Perveen

Bismillah Taqee Institute of Health Sci., KARACHI, Pakistan

Backgrounds. PRC transfusion and iron chelation remains the mainstay of treatment of β-thalassaemia major patients. HbF augmentation is the exciting new approach to treat haemoglobinopathy. *Aims.* This study evaluates the efficacy and safety of HU to reduce the volume of PRC transfusion in β-thalassaemia. *Methods.* 23 patients with β-thalassaemia major received HU mean dose, 16mg/kg/day. The results were analyzed at the end of 24 months. Transfusion requirement 6 months before starting HU was considered as control. *Results.* 20 patients were evaluable after 24 months. Mean volume of PRC transfused was reduced in all. Mean PRC requirements for six months before starting HU was 2126.45 mL where as after 24 months on HU was 1489.59 ml (mean difference: 637.3 mL; 95% CI: 402.8 - 817.8; p<0.001). Interval between transfusions was increased by 68.7%. Mean increase was 12.1 days (CI: -18.0, -6.3; p value: <0.001). Statistically insignificant increase was noted in ferritin levels with mean difference of 657.1 ng/L (95% CI: -1475.3, 161.1; p-value: 0.1). Grade I myelosuppression was seen in four and diarrhea in two patients. *Conclusion*. Hydroxyurea was found to be a safe medicine in β-thalassaemia. It showed a reduction in transfusion requirement and increased interval between PRC transfusions.

1135

ENDOGENOUS ERYTHROPOIETIN PRODUCTION AND ERYTHROPOIETIC ACTIVITY IN ANEMIC CANCER PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

G. Kostova,[1] S.N. Siljanovski,[2] G.B. Georgievski[2]

[1]*Clinic for hematology, SKOPJE, Macedonia;* [2]*Clinic for Hematology, SKOPJE, Macedonia*

Backgrounds. Cancer anemia is multifactorial: blunted erythropoietin

(Epo) response has also been encountered. As it is particularly well documented only in some types of anemia of chronic disease (ACD), we investigated the Epo deficiency in anemic cancer patients with hematologic malignancies. Patients and Methods. 42 patients (pts) with multiple myeloma (MM), 27 pts with malignant lymphoma (ML) and 19 pts with chronic lymphocytic leukemia (CLL) were included in the study. 25 pts with iron deficiency anemia represented the control group. Serum Epo and serum transferin receptors (sTfR) were measured with commercially available assays. O/PEpo and O/PsTfR ratios (O-observed value, P-predicted value) were derived for each patient in order to asses if Epo response and erythropoietic activity are appropriate for a given degree of anemia. Predicted values were calculated from the regression equation of serum Epo, respectively sTfR versus hemoglobin (Hb) determined in the control group. A correlation between O/PEpo and O/PsTfR was searched in order to asses the impact of Epo deficiency on erythropoietic activity and therefore the anemia. *Results.* All pts, except for the MM pts with renal failure showed Epo response to anemia: a significant inverse correlation between serum Epo and Hb was found. Epo production and erythropoietic activity as determined from the control group were inappropriate if the values for O/PEpo and O/PsTfR were ≤0.8 and 0.9, respectively. 43% pts with MM and no renal failure, 33% pts with ML and 11%pts with CLL had inappropriate Epo response to anemia. Erythropoietic activity was inappropriate in 76% pts with MM, 48% pts with ML and 37% with CLL. The inappropriate erythropoietic activity and therefore the anemia were significantly influenced from the inappropriate erythropoietin production: this could be shown by the positive correlation between O/PEpo and O/PsTfR in all three patient groups. *Conclusion*. Significant number anemic pts with hematologic malignancies have blunted Epo response to anemia. The adequacy of serum Epo levels could be convincingly assessed by O/PEpo and O/PsTfR ratio and should be used to predict the therapy response to rHuEpo in anemic cancer pts.

1136

THE SAFETY OF AVOIDING PRE-OPERATIVE TRANSFUSION IN PATIENTS WITH SICKLE CELL ANEMIA

S.K. Al Jaouni, M. Al Muhayawi, H. Qari

King Abdulaziz University, JEDDAH, Saudi Arabia

Backgrounds. Sickle cell anemia (SCA) is a common heredity blood disease seen in Saudi Arabia, the affected patients associated with severe clinical manifestations. It is generally recommended for patients with sickle cell anemia to receive red blood cell (RBC) transfusions before undergoing general anesthesia and surgery. Patients with sickle cell anemia have increased chance of undergoing surgical procedures with higher morbidity. The practice of preoperative blood transfusion for such patients is still controversial. Lately, a great deal of controversial data accumulated in regards to transfusion management of such patients who require surgery. *Aim*. The aim of this prospective study was to assess the role of pre-operative transfusion practice in patients with SCA, whether or not omissions of such preparation lead to complications.

Table 1. Illustrates the post-operative complications in both groups of SCA.

Complications	*Group I (N=181)*	*Group II (N=188)*	*p Value*
Painful Crises	3	5	
Neurological Complication	0	4*	
Minor Respiratory Complication	5	4	
Respiratory Distress (Atelectasis)	0	3*	
Circulatory Overload or Heart failure	0	5*	
Infection	3	3	
Others	2	3	
Total (Percentage)	13 (7.0%)	27 (14.0%)	0.002
Dealy of Surgery	1	45**	> 0.001

** Hemoglobin raised ≥ 10.5 g/dL; ** To fulfill the criteria of Hb S concentration ≤ 40% pre-operative.*

Methods. A randomized study of 369 patients, median age 16-years-old (range: 1-35 years old), during the period between June 1996 and June 2001, underwent different surgical procedures at King Abdulaziz University Hospital and King Fahd Armed Forces Hospital. Surgical procedures included adenoidectomy, tonsillectomy, total hip arthroplasty, cholecystectomy, splenectomy, and Obstetric and Gynecological surgeries. Patients were randomized into two groups: Group I (n=181), received no preoperative transfusion but were transfused compensated for blood loss during surgery. Group II (n=188) received simple or partial exchange transfusion preoperatively. All patients were clinically and hematological stable in the immediate pre-operative period; also, were carefully hydrated and good oxygenation was maintained. *Results.* Results showed none of the patients developed major intra- or post-operative complications in both groups. 14.4% of the preoperative transfusion group developed post-operative complications in comparison to 7.2% in non-transfused group with a significant *p* value (0.002). *Conclusion*. Avoidance of preoperative transfusion is a safe practice in properly selected steady state sicklers. On the contrary, it is believed that the risks associated with transfusion were avoided.

1137

PHARMACOKINETICS OF ERYTHROPOIETIN PRODUCED BY A HUMAN CELL LINE (EPOETIN Δ): SUBCUTANEOUS VS. INTRAVENOUS DOSING IN PATIENTS WITH CHRONIC KIDNEY DISEASE

R. Pratt

Shire Pharmaceuticals, WAYNE, USA

Backgrounds. Epoetin Δ (Dynepo™, Shire) is an erythropoietin produced by gene-activation technology in a human cell line. As a result, it contains very few of the highly immunogenic Neu5Gc residues that are more commonly found in other recombinant erythropoietins. In renal anaemia, the preferred route of administration for erythropoietin (either subcutaneous [sc] or intravenous [iv]) often differs depending on the status of the patient, the erythropoietin used and local practice. Aims. To assess the pharmacokinetics of iv or sc epoetin delta in patients with anaemia and end-stage renal disease requiring dialysis. Methods. Patients with end-stage renal disease requiring dialysis who had been receiving epoetin α for at least 90 days entered a 1-week washout phase during which they did not receive recombinant erythropoietin. Patients were then randomized to one of four groups receiving single doses of epoetin delta 150 IU/kg (iv or sc) or 300 IU/kg (iv or sc). Blood samples were drawn before and for 72 h after administration. Results. In total, 28 patients entered the washout phase and 22 of these (12 men, 10 women) went on to receive epoetin Δ and complete the study. Pharmacokinetic parameters are shown in Table 1.

Intravenous						
Dose (U/kg)		AUC_{inf} (h.EU/L)	Cmax (EU/L)	CL (L/h/kg)	V_z (L/kg)	$t_{1/2}$
150	N (obs)	5	5	5	5	5
	Mean	36208	3257.2	0.007	0.097	9.9
	CV%	33.6	31.5	43.5	38.2	8.0
300	N (obs)	5	5	5	5	5
	Mean	77736	4769.9	0.005	0.097	13.2
	CV%	18.9	43.4	29.7	28.7	22.2
Subcutaneous						
150	N (obs)	6	6	6	6	6
	Mean	9547	162.2	0.026	1.28	33.1
	CV%	42	63	37	46	40
300	N (obs)	6	6	6	6	6
	Mean	27888	467.7	0.020	0.78	27.8
	CV%	43	40	56	60	27

Bioavailability of sc epoetin Δ was 26'36% of that of iv epoetin Δ. The half-life of sc epoetin delta was approximately 30 h compared with 10'13 h for iv epoetin Δ Treatment-emergent adverse events occurred in 45% of patients, but none of these were considered by investigators to have any relation to epoetin Δ. *Conclusions.* As expected, the pharmacokinetics of epoetin Δ differ depending on route of administration. The half-life of epoetin Δ in patients with renal anaemia may be slightly higher than that reported for epoetin alfa (a half-life as low as 4 h has been reported), suggesting that longer dosing intervals may be possible with this agent.

1138

TWO NOVEL G6PD VARIANTS, G6PD PEDOPLIS-CKARO AND G6PD PIOTRKOW IN POLAND

M. Maciag,[1] E. Mendek Czajkowska,[2] D. Plochocka,[1] E. Zdebska,[2] E. Jablonska Skwiecinska,[1] I. Witos,[2] T. Urasinski,[3] B. Burzynska[1]

[1]*Institute of Biochemistry and Biophysics, WARSAW, Poland;* [2]*Inst. of Haematology & Blood Transfusion, WARSAW, Poland;* [3]*Pomeranian Medical University, SZCZECIN, Poland*

Backgrounds. Glucose-6-phosphate dehydrogenase (G6PD) is the key enzyme of the pentose phosphate pathway whose main physiological function in red blood cells is to produce the NADPH, essential for the protection of the cells against oxidative stress. The majority of people with G6PD deficiency are asymptomatic but they may develop acute hemolytic anaemia in association with infections or following the ingestion of certain drugs or fava beans. In some sporadic cases G6PD deficiency is the cause of chronic non-spherocytic haemolytic anemia, CNSHA. *Aims.* The aim of our study was to elucidate the molecular basis of G6PD deficiency. *Methods.* Genomic DNA was extracted from peripheral blood using standard methods. G6PD gene exons 2 to 13 were amplified by polymerase chain reaction (PCR). DNA fragments generated by PCR amplification were directly sequenced. The appropriate restriction enzyme analysis was used to verify the presence of found mutations. Molecular modeling of the tertiary structure of the G6PD molecule was used to check the influence of these mutations on the enzyme structure. *Results.* Direct sequencing of G6PD gene of two compensated G6PD-deficient patients showed two novel mutations: 573C→G mutation which is located in exon 6 and results in the substitution F191L and 851T→C substitution that predicts valine to alanine at the position 284. F191 does not belong to the highly conserved residues. Surrounding of this residue is hydrophobic, so replacement of the Phe by the not much smaller Leu should not affect the overall protein fold. It has been confirmed by the energy minimization calculations for the wild type enzyme and F191L mutant. The V284A substitution neither change polarity of the amino acid residue nor introduce steric hindrance. Comparison of the minimized structures of the wild type and V284A mutant showed minor changes in the course of the main chain and no changes in the positions of the residues forming salt bridges, so this mutation does not cause serious structure distortions. *Conclusions.* Molecular analysis revealed the presence of two new G6PD variants F191L and V284A which were named G6PD Pedoplis-Ckaro and G6PD Piotrkow respectively, after the patients' place of origin. The results of molecular modeling correlate with clinical picture of studied patients.

1139

BASELINE IRON STUDIES DEMONSTRATE SEVERE IRON OVERLOAD IN PATIENTS ENROLLED INTO THE DEFERASIROX (EXJADE, ICL670) CLINICAL TRIAL PROGRAMME

C. Kattamis,[1] B. Meddeb,[2] C. Ressayre-Djaffer,[3] T.A. Balakina[4]

[1]*University of Athens Medical School, ATHENS, Greece;* [2]*Hpital Aziza Othmana, TUNIS, Tunisia;* [3]*Novartis Pharma AG, BASEL, Switzerland;* [4]*Hematological Scientific Centre, MOSCOW, Russian Federation*

Backgrounds. Iron overload is a potentially life-threatening consequence in regularly transfused patients with chronic anaemias. Although it can be effectively managed with iron chelation therapy many patients are currently inadequately chelated, mainly because of the demanding administration regimen of the reference standard chelator deferoxamine (DFO, Desferal®), and therefore do not gain the full benefits of treatment. Deferasirox (Exjade®, ICL670) is a novel, once-daily, easily applicable, oral iron chelator that is currently approved for use in eight countries, including the USA and Switzerland, in patients aged ≥2 years with chronic transfusional iron overload. To date, more than 1,000 patients have been enrolled into the deferasirox clinical trial programme. Aims. The primary aim of this cross-study analysis was to evaluate the severity of iron burden in patients prior to enrolment into the deferasirox clinical trial programme. *Methods.* Liver biopsy was performed at baseline and after 1 year in patients participating in two deferasirox clinical studies, 107 (n=495) and 108 (n=120). In Study 107, 248 patients with β-thalassemia were randomized to deferasirox (5, 10, 20 or 30 mg/kg/day) and 247 to DFO (<25, 25'<35, 35'<50 and ≥50 mg/kg) according to baseline liver iron concentration (LIC). In Study 108, 67 patients with β-thalassemia and 53 with other anaemias (eg myelodysplastic syndromes, Diamond-Blackfan anaemia, rare anaemias) were enrolled and received deferasirox. The enrolled patients originated from 12 countries (Argentina, Belgium, Brazil, Canada, Germany, France, Greece, Italy, Tunisia, Turkey, UK, USA). Results. In general, baseline characteristics were comparable between deferasirox and DFO cohorts. Paediatric patients (aged <16 years) comprised 38% of the study population. Mean baseline LIC was high in the overall population (Table 1), with approximately 80% of patients having a baseline LIC ≥7 mg Fe/g dw and the majority (68.6%) ≥10 mg Fe/g dw. Published data have linked LIC levels above >7 mg Fe/g dw with an increased risk of developing iron overload-related complications, primarily heart-related. Baseline serum ferritin levels were also high and above clinically acceptable values. For most patients, transfusional iron intake was 0.3±0.5 mg/kg/day, corresponding to a mean daily amount of blood given of 0.35 ± 0.11 mL RBC/kg. In addition to this global analysis, local analyses by country have been performed. *Conclusions.* Baseline iron burden, as reflected by LIC and serum ferritin levels, was very high and above published clinically acceptable thresholds. This analysis demonstrates that despite the availability of chelation therapy, many patients were severely iron overloaded and therefore at high risk for developing complications. There were no differences between adult and paediatric patients. This suggests that patients were not receiving adequate chelation therapy to achieve iron balance. The development of a highly efficacious, well-tolerated and convenient iron chelator will improve compliance and allow physicians to use an effective chelation programme for their patients. This was a primary goal of the rigorous development programme for the once-daily, oral chelator deferasirox, which culminated in its registration with a broad indication by a number of health authorities.

Table 1. Patient demographics and baseline characteristics.

	All patients (n=615)
Median age, years	19.0
Patients aged 2 to <16 years, n(%)	213 (37.6)
Patients aged ≥16 years, n(%)	384 (62.4)
Male: Female	299:316
Mean baseline LIC±SD, mg Fe/g dw	
All patients	16.4±10.4
2 to to <16 years	16.4±10.0
≥16 years	16.4±10.6
Baseline LIC category, n(%)	
<7 mg Fe/g dw	127 (20.7)
7 to <10 mg Fe/g dw	66 (10.7)
≥10 mg Fe/g dw	422 (68.6)
Mean baseline serum ferritin ±SD, ng/mL	
All patients	2898±2099
2 to to <16 years	2870±1662
≥16 years	2914±2325
Mean transfusional iron intake ±SD, mg/kg/day	0.38±0.12
Iron intake category n (%)	
0	5 (0.8)
0 to <0.3 mg/kg/day	153 (24.9)
0.3-0.5 mg/kg/day	357 (58.1)
>0.5 mg/kg/day	100 (16.3)
Mean blood given ±SD, mL RBC/kg/day	0.35±0.11

1140

DETECTION OF RARE RHCE ALLELES IN COMORIAN INIVIDUALS LIVING IN MARSEILLES, FRANCE

M. Touinssi, P. Bailly, J. Chiaroni

EFS-Alpes Mditerrane, MARSEILLE, France

Background. More than 70 000 comorians are living in Marseilles at present (10% of the total Comorian population has immigrated to France since 1970). Due to their genetic background and lack of data on this population, some difficulties of transfusion are encountered. As described previously (*Noizat et al. Blood, 2002*), some rare RHCE phenotypes are found exclusively in black populations: i) RH : -18 (712A→G) with the three alleles (ceEK, ceAR, ceBI), ii) RH : -34 phenotype is produced by the (C)ces haplotype, iii) Partial Rhe : produced by the new *ces(340)* allele carrying an extra-mutation in exon 3 (340C→T) and by the ceMO allele. *Aims* :The aim of this work was to detect rare RHCE alleles, in Comorians of Marseilles, and to supply data on this population. A cohort of 260 unrelated immigrants of both sexes living in Marseilles France, participated to this study and were considered as representative of Comorian population, particularly, Grande Comore. This study was approved by the competent authorities in France (CCPPRB n°00/43). Methods. Genomic DNA was isolated from peripheral blood leukocytes using a Qiamp Blood DNA Mini kit (Qiagen®, Hilden, Germany). Allele-specif-

ic primer PCRs were used to detect specific mutations corresponding to RHCE rare alleles and to determine their homozygous or heterozygous status. *Results*: Six individuals were found positive for the 712A→G mutation. Sequencing of RHCE exons 4, 5 and 6 showed that five individuals were heterozygous for ceAR and one was heterozygous for ceKE. 733G→C mutation, associated with antigens RH10 and RH20, was observed with high frequency (154 were positive and 58 were homozygous for this mutation). Twenty individuals carried hybrid D-CE(3-8)-D gene heterozygously, future sequencing will show if this hybrid gene, found in our Comorian population of Marseilles, is similar to the one found in African populations. Detection of 340C→T is still under study. Nine individuals carried ceMO heterozygously as they carried both T667 mutation and the wild-type G667. Furthermore, a new 902A→G (Asp301Gly) was detected and appeared to be specific to this Comorian population. The effect due to this particular mutation, which could be considerd as a new RHCE variant, is not known yet. *Conclusions.* These preliminary findings allowed us to calculate the incidence of the rare RHCE alleles and haplotypes in our population of 260 Comorian individuals as follows: ceEK :0.38%; ceAR: 1.92%; no ceBI was found, ceMO: 3.46%; (C)ces seemed to be present with a high frequency. This study showed that a variety of RHCE alleles are present in Comorian population of Marseilles. These data should contribute to define a particular strategy for transfusion in black populations.

Acknowledgments. We are grateful to F. Noizat-Pirenne, MD, (EFS-Ile de France Paris, France) for her technical advices, and to Mr K. PAPA for referring to our laboratory the donor samples.

1141

A NEW SMALL PKLR GENE DELETION ASSOCIATED WITH A MISSENSE MUTATION IN A PATIENT WITH PYRUVATE KINASE DEFICIENCY AND COMPENSATED HEMOLYSIS

L. Manco, L. Relvas, U. Rebelo, J. Vidán, M.L. Ribeiro

Centro Hospitalar de Coimbra, COIMBRA, Portugal

Background. Pyruvate kinase (PK; EC 2.7.1.40) deficiency is the most common enzyme abnormality in the erythrocyte glycolytic pathway causing chronic nonspherocytic hemolytic anemia. The PK deficient anemia, transmitted as an autosomal recessive disorder, has a heterogeneous clinical phenotype, ranging from a mild chronic hemolysis to a severe anemia presenting at birth and requiring exchange transfusion. To date more than 130 PKLR mutations have been identified most of them missense mutations; only eleven are small deletions. *Aims*. The aim of the study was to understand the genotype/phenotype correlation in a PK deficient patient with compensated hemolysis. *Patient*. A Portuguese child was diagnosed with PK deficient anemia at the age of 2 months. Her spleen was not palpable and her hematological parameters were Hb=74g/L, Htc=21%, MCV=88fL, MCH=31pg, MCHC=35g/dL, RDW=14.6%, reticulocytes= 6.4%, WBC=5900/µL, Plt=668000/microL, unconjugated bilirubin=26.3 µmol/L (N<17 µmol/L). PK activity was 15.7% of normal. At the age of 14 months she got fever, splenomegaly and severe anemia (Hb=46g/L) and was diagnosed with Kalazar. A complete recover was reached after 3 months treatment with Glucantim. Nowadays she is 7 years old, has a normal global development, no palpable spleen, infrequent and self-limited hemolytic crisis, normal Hb levels (13.5g/dL), slight reticulocytosis (1.8%), unconjugated bilirubin=11µmol/L and PK activity was 11.8% of normal. Methods. After informed consent, genomic DNA was extracted from an EDTA peripheral blood sample and PKLR gene was studied by PCR, SSCP and sequencing analysis. *Results*. SSCP mobility shifts were detected in two PCR fragments spanning PKLR exons 3 and 8. Automatic sequencing revealed 2 mutations: a 993C→A substitution (exon 8), predicting the amino acid substitution 331Asp→Glu, which has been previously described in association with PK deficiency; and a 22 bp deletion (exon 3) involving the cDNA nucleotides 109 to 130. *Conclusions*. The Asp331 is an internal residue located in the A domain of the PK subunit, between alfa5-helice and β6-strand, a highly conserved region close to the PEP binding site and involved in the PK subunit transition from the low substrate affinity T-state to the high affinity R-state. Translation of the other allele carrying the del 109-130 mutation is predicted to result in a short mRNA, interrupted by a stop codon (...AACTGA) 3 triplets downstream the deletion. This mRNA may be unstable or codes for a truncated R-PK subunit 39 amino acids long, lacking more than 90% of the normal amino acid sequence at the COOH-terminal. It is interesting to notice that, despite patient R-PK enzyme activity most probably results from a catalytically severely affected Asp331Glu tetramer, the patient has a well compensated hemolysis, with normal steady state hemoglobin levels.

1142

MEASUREMENT OF ERYTHROCYTE BAND3 EXPRESSION IN HEREDITARY SPHEROCYTOSIS

S. Jacobsson, H. Johansson, G.L. Persson

Sahlgrenska University Hospital, GOTHENBURG, Sweden

Backgrounds. Hereditary spherocytosis (HS) is the most common inherited anemia among the spherocytic anaemias, whereas autoimmune hemolytic anemia is the most common acquired. The prevalence of HS is not known, partly because of the heterogenous clinical manifestations. The most specific diagnostic techniques, i.e. erythrocyte protein analysis and molecular genetics are only provided by a few reference laboratories. In the routine setting diagnosis is based on typical family history, splenomegaly and jaundice and the finding of spherocytes and reticulosis in the blood and increased osmotic fragility of the erythrocytes. The red cell anione exchanger band 3 is present in gross numbers in the erythrocyte cell membrane. In HS the expression of band 3 is diminished irrespective of the primary protein defect. The band 3 expression can readily be measured by flow cytometry after labelling with eosin-5-maleimide (EMA) (*King et al. 1999*). *Aim*. Determine the diagnostic characteristics of the flow cytometric band 3 expression test. *Methods.* We have measured band 3 expression in 50 patients with HS, 50 patients with other hemolytic anemias and in 200 healthy volunteers. We have also studied band 3 expression in patients with autoimmune hemolytic anemia, G-6-PD-defiency, PK-deficiency, sickle cell anemia and other rare forms of anemia. We have also studied the influence of recent transfusions and ongoing profuse hemolysis. *Results.* In our laboratory the cut off value for band 3 expression for diagnosing HS is 92,5% of that in non-HS persons. We confirm the findings of others that the band 3 expression is normal in all other forms of anaemia than HS. Ongoing hemolysis and recent transfusions can diminish the sensitivity of the method. *Conclusion.* The flow cytometric measurement of band 3 expression has a high sensitivity and specificity for diagnosing hereditary spherocytosis and should be one of the primary investigations in cases of suspected hereditary spherocytosis.

1143

GLUCOSE TOLERANCE IN PATIENTS WITH β-THALASSAEMIA MAJOR

N. Angelopoulos,[1] S. Livadas,[2] A. Zervas,[1] M. Noutsou,[3] G. Rombopoulos,[1] E. Katounda,[1] G. Tolis,[1] V. Kaltzidou,[1] D. Kaltsas[1]

[1]*Hippocratio Hospital, ATHENS, Greece;* [2]*Endocrine Unit, 'Ag.Sophia' Hospital, ATHENS, Greece;* [3]*Diabetes Department,Hippocratio, ATHENS, Greece*

Backgrounds. In chronically transfused thalassemic patients, whether insulin resistance is the primary abnormality leading to glucose metabolism disturbances, or early introduced pancreatic damage and reduced insulin secretory capacity is the main cause of glucose intolerance, remains uncertain. *Aims.* To examine the incidence of glucose disturbances and endocrine co-morbidity in transfused patients with β-thalassaemia major. *Methods*. We assessed glucose responses during an oral glucose tolerance test in 243 regularly transfused thalassaemic patients (107 males, 136 females, 25.26 ±6.2 years, age ± standard deviation, range 13-48). Patients' records were thoroughly reviewed to determine the overall transfusional iron overload and start of chelation therapy (age and time of first blood transfusion and start of iron chelation). *Results.* Patients were stratified according to the criteria of the American Diabetes Association in three subgroups: Normal glucose tolerance (NGT, n=197,81%) impaired glucose tolerance (IGT, n=25, 10,3%) and diabetes (n=21, 8,7%).There were no differences between the groups regarding the parameters of transfusion/Chelation therapy. However, the development of glucose intolerance was significantly increasing with age (p<0.001). Except hypothyroidism, all the other endocrine complications were significantly more frequent in patients with diabetes and IGT (Table).

	Cardiopathy	*Hypothyroidism*	*Hypoparathyroidism*	*Hypothyroidism*	*Osteoporosis*
NGT N=197 81%	12,18% (24/197)	10,66% (21/197)	65,48% (129/197)	28,42% (56/197)	37,56% (74/197)
IGT N=25 10,3%	36% (9/25)	28% (7/25)	92% (23/25)	36% (9/25)	44% (11/25)
Diabetes N=21 8,7%	38,09% (8/21)	23,81% (5/21)	42,85%	33,33% (7/21)	57,14%
Fisher's Exact Test	p<0.001	p=0.0015	p=0.0017	p=0.6	p=0.0013

Conclusions. The degree of iron overload, at least as this can be reflected by ferritin levels, is not associated with the development of glucose intolerance. Long-term iron balance rather than the present iron status seems to be related to the development of glucose metabolic disorders. Physicians caring for patients with thalassaemia major should be particularly alert to glucose intolerance since co-existence of other endocrine complications is common in these patients.

1144

EVOLUTION OF THROMBOCYTOSIS RELATED TO IRON DEFICIENCY ANEMIA

M. Laatiri,[1] M. Bedoui,[2] K. Zahra,[1] M. Kortas,[1] A. Kelaf[1]

[1]*Hopital Farhat Hached, SOUSSE, Tunisia;* [2]*Hopital Farhat Hached, SOUSSE, Tunisia*

Backgrounds. The incidence and the outcome of thrombocytosis related to iron deficiency anemia is not described very well. The aim of this study was to perform an analysis of the evolution of thrombocytosis related to iron deficiency. We performed a retrospective analysis of 1570 consecutive patients with iron deficiency anemia collected between 1995 and 2005. Four hundred 40 patients (29%) had a thrombocytosis more than 500×10^9/L. They were between 18 months and 85 years old. The mean of hemoglobin was 53 g/l and the mean of platelets was 745×10^9/L. There was no correlation between the importance of anemia, iron deficiency and the importance of thrombocytosis. The evolution was favourable in all cases with correction of thrombocytosis with a median of 16 days. There was not any thrombo-embolic complication. We conclude that thrombocytosis is frequently associated to iron deficiency anemia and the outcome is always fovourable with a rapid correction and without risque of thrombo-embolism.

1145

EFFICACY OF IRON-CHELATION THERAPY IN BULGARIAN CHILDREN WITH β-THALASSEMIA MAJOR

K. Dimitrova,[1] D. Stoyanova,[1] I. Kalev,[2] V. Spasov[1]

[1]*Thalassemia Centre, Children`s Universit, SOFIA, Bulgaria;* [2]*Aleksandrovska Hospital, SOFIA, Bulgaria*

Backgrounds. Iron-chelation therapy is an essential part of clinical management of patients with β-thalassemia major (TM). It decreases iron-induced organ damage and provides longer survival of thalassemics. Deferoxamine (DFO) and deferiprone (L1) are two iron chelators available now in most of the countries. *Aims.* To evaluate the efficacy and safety of iron-chelation therapy in Bulgarian pediatric patients with TM. *Methods.* 35 children with TM (age 3-16 years) were included. They were treated with DFO alone (DFO administered subcutaneously 40 mg/kg 5 days/week in 30 children) or DFO in combination with L1 (DFO subcutaneously 40 mg/kg 3 days/week with L1 orally 75mg/kg/d in five children). Serum ferritin (SF) concentration was used as a marker for efficacy of iron-chelation therapy. Data on SF levels collected from all participants for a three-years period (January 2003-January 2006) were retrospectively analysed. SF concentration was measured every three-four months. SF levels less than 2500 ng/mL (2500 mcg/L) indicated effective chelation therapy, whereas higher SF concentrations indicated ineffective treatment. *Results.* DFO alone was found ineffective in 9/30 patients (30%) mainly due to poor treatment compliance. Combination therapy demonstrated inefficacy in one child, 1/5, (20%). No serious adverse events associated with DFO or L1 were recorded. *Conclusions.* In patients with good treatment compliance, DFO is effective in reducing iron overload. For thalassemics with poor compliance, more flexible approach, including combination therapy, should be considered.

1146

UNRELATED STEM CELL TRANSPLANTATION FOR CHILDRENS β-THALASSEMIA MAJOR IN CHINA

F. Feng

Nanfang Hospital, GUANGZHOU, China

Backgrounds. Allo-genetic stem cell transplantation(SCT) is the only way to cure β-thalassemia major in present. But most children with β-thalassemia major have no chance of undergoing stem cells transplantation due to lack of matched sibling because of family policy in China *Aims.* Unrelated SCT had been studied to Chinese children with β-thalassemia major to explore it's method and effects in China. *Methods.* Eleven children of β-thalassemia major had undergone unrelated SCT between Jan. 2005 and Dec. 2005. Six recipient/donor pairs are 6/6 HLA(A,B,DRB1) high resolution typing matched and five recipient/donor pairs are 1/6 HLA high resolution typing mismatched. Six children received peripheral blood stem cells transplantation (PBSCT) and 6 children received bone marrow transplantation (BMT). β-thalassemia major children conditioned with BU 14-21 mg/kg+CY(140-200 mg/kg)+ ATG(25-35mg/kg)+Flu(200 mg/m^2). The graft contained median mononuclear cells 5.3×10^8/kg(rang, 2.2×10^8/kg -10.0×10^8/kg) and median $CD34^+$ cells 6.6×10^6/kg(rang, 1.4×10^6/kg -22.5×10^6/kg). All patients received CSA FK506+ MMF(MTX) as graft versus host disease (GVHD) prophylaxis and heparin + pGE1 as veno-occlusive disease (VOD) prophylaxis. *Results.* All of 11 children had got complete chimerism by the identification of FISH for XY chromosome or quantitative PCR for short tandem repeat (STR) and 10 /11(91%) children are alive without thalassaemia after a median follow-up time of 5.8 months after transplant (range, 2-13 months). One child(9%) died from IV degrees acute GVHD. The ANC engrafted from +9 to +16 days and the time of platelets>20×10^9/L was from +11 to +50 days. Five of eleven(45%) children suffered I-II degrees acute GVHD and 2/11 (18%)children (all underwent PBSCT) suffered I-II degrees acute GVHD. Complications included 2 case of infection in central nervous system(1 case of virus and 1 case of bacteria) and 4/11 (36%)cases of mild VOD. *Conclusions.* Our study showed higher incidence of severe aGVHD in unrelated PBSCT for children with thalassemia major. Unrelated SCT can be alternative effective therapy for children's β-thalassemia major in China

1147

PRETREATMENT WITH DASATINIB HAS NO ADVERSE INFLUENCE ON TRANSPLANT OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR PH+ CHRONIC MYELOGENOUS LEUKEMIA AND ACUTE LYMPHOBLASTIC LEUKEMIA

M. Leiba, A. Shimoni, I. Hardan, R. Yerushalmi, A. Nagler

Chaim Sheba Medical Center, TEL HASHOMER, Israel

Allogeneic stem cell transplantation (alloSCT) is frequently used as salvage or curative therapy in patients with advanced chronic myelogenous leukemia (CML) or Ph+ acute lymphoblastic leukemia (ALL) who were previously treated with Imatinib. While one study showed higher incidence of GVHD, VOD and TRM, most studies have demonstrated that Imatinib therapy prior to alloSCT does not adversely affect transplantation outcome. Dasatinib is a novel dual SRC/ABL kinase inhibitor currently being used in patients with Imatinib-resistant advanced CML or relapsed/refractory Ph+ ALL. Most of these patients will eventually undergo alloSCT, raising the question of whether Dasatinib therapy may adversely affect transplantation outcome. We report three patients with advanced CML (n=2) and Ph+ ALL (n=1) who received Dasatinib prior to alloSCT from an HLA matched sibling (n=2) or mismatched related donor (HaploCT) (n=1). All were male with a median age of 28 (16 to 49) years. All three achieved complete hematological response, as well as complete (n=2) or partial (n=1) cytogenetic response prior to transplantation. They were conditioned with either a myeloablative protocol (n=2) or a reduced intensity protocol (n=1). GVHD prophylaxis consisted of CSA and MTX (n=2) or complete T cell depletion (n=1). Each patient received a mobilized peripheral blood stem cell graft with between 11.4 to 19.8 CD34+ cells/kg. All patients successfully engrafted reaching ANC > 0.5×10^9/L on median day +11 and plt > 20×10^9/L on median day +11. No patient developed unusual organ toxicities including no hyperbilirubinemia or VOD. There was no increased risk of infection in the sibling transplants. No patient developed clinically significant GVHD. In this small number of patients with advanced CML and Ph+ ALL (the first to be reported receiving an alloSCT following Dasatinib therapy), we found no evidence that Dasatinib adversely affect post alloSCT outcome. Larger studies are obviously indicated to confirm our preliminary results.

1148

ADDITION OF CYTARABINE DOES NOT IMPROVE UPON MOLECULAR RESPONSES ACHIEVED BY IMATINIB ALONE IN CHRONIC MYELOID LEUKEMIA

P. Mishra, S. Sazawal, M. Mahapatra, R. Saxena, D.R. Choudhary, A. Dixit, T. Chatterjee, R. Kumar, V.P. Choudhry

AIIMS, NEW DELHI, India

Backgrounds. The introduction of Imatinib, a targeted therapy for chronic myeloid leukemia (CML) has been one of the success stories of modern cancer management. However molecular techniques have shown that only 4-10% of Imatinib treated patients achieve complete molecular remission. *Aim.* To compare Imatinib alone with Imatinib - cytarabine to see if the combination could achieve greater molecular

responses. Methods. 85 newly diagnosed adult CML patients were randomized to receive Imatinib or Imatinib-cytarabine combination. Only those patients in chronic phase were included in the study. Hydroxyurea was the only therapy received by these patients prior to starting Imatinib. Imatinib was initiated at a dose of 400 milligrams, within 6 months of diagnosis. Cytarabine was added in 45 patients at a dose of 10 milligram /square meter for 10 days every month after 3 months of Imatinib therapy. BCR-ABL transcripts were measured by real time PCR at baseline and at follow-up every 6 months. The reduction in BCR-ABL transcripts in the two groups was compared using Mann-Whitney test. Results. Patients were followed for a median period of 1.5 years (range 0.6-2 years) in the Imatinib group and 1.8 years (range 1-2 years) in the Imatinib-cytarabine group. The baseline variables like age, Sokal risk groups and haemogram were similar in the two groups. Median values for age, haemoglobin, total leukocyte count and platelet count in the Imatinib group were 31 years (range 21-45 years), 11gm% (range 5.6-15.4 gm%), 150000/cubic millimeter (range 112000-242000/cubic millimeter) and 540000/cubic millimeter (range 125000-1126000/cubic millimeter) respectively. Median values for age, haemoglobin, total leukocyte count and platelet count in the combination group were 33 years (range 22-55 years), 11gm% (range 7.6-15.8 gm%), 128000/cubic millimeter (range 120000-205000/cubic millimeter) and 538000/cubic millimeter (range 159000-1032000/cubic millimeter) respectively. Male: Female ratio was 3:1 in Imatinib group and 5:1 in the combination group. All patients achieved complete haematological responses. Both groups tolerated their therapies equally well with no significant difference in toxicity profiles. 2 patients in each group discontinued therapy because of grade 4 cytopenias. 1 patient in the Imatinib group and 2 patients in the combination group discontinued therapy because of grade 4 skin toxicities. In the Imatinib group the median number of BCR-ABL transcripts at diagnosis was 345808 (range 605-10125616), which had reduced to 18286 (range 0- 5412661) at follow-up, a median log reduction of 1.255 (range 0.975-4.02). In the combination arm, the median number of BCR-ABL transcripts at diagnosis was 132498 (range 2712-12122832), which had reduced to 15415 (range 4-4328466) at follow-up, a median log reduction of 1.305 (range 0.12-8.2). This reduction in BCR-ABL transcripts in the two groups was not statistically significant (p=0.945). 2 patients in each group achieved more than 3 log reduction in BCR-ABL transcripts. *Conclusion*: We conclude that addition of cytarabine does not improve significantly upon the molecular responses seen with imatanib alone.

1149

CLINICAL SIGNIFICANCE OF QUANTITATIVE REAL-TIME PCR FOR MONITORING OF MINIMAL RESIDUAL DISEASE FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE RECEIVING GLIVEC THERAPY

E.Y. Chelysheva, A.G. Turkina, A.V. Misyurin, E.V. Aksenova, E.V. Domracheva, A.V. Zakharova, N.D. Khoroshko

RAMS, MOSCOW, Russian Federation

Background. With the high possibility of receiving major cytogenetic response (MCR) and complete cytogenetic response (CCR) for patients with chronic myeloid leukemia (CML) receiving Glivec therapy it is more significant to study minimal residual disease with the help of more sensitive methods than standart cytogenetic analyses. Real-time PCR is a specific and suitable method for quantitative characteristic of molecular response for CML patients treated with Glivec. *Aim*. Search of prognosticaly significant levels of minimal residual disease for CML patients in chronic phase (CP) treated with Glivec. *Patients and methods*. We have analysed 105 samples of peripheral blood and bone marrow and estimated a molecular response (MR) of 53 CML CP patients with MCR and CCR receiving Glivec therapy 400 mg daily after interpherone-α treatment failure. Median of observation was 36 months (6-54 months). We analysed levels of BCR-ABL™ 210 transcript by quantitative Real-time PCR, TaqMan technology (ICycler IQ). β2 microglobuline was used as housekeeping gene. Results were expressed as ratio BCR-ABL/b2 microglobuline x 10^8. We also assessed the lg difference of baseline and the results. The baseline in our investigation has been established according to the analyses of diagnostic levels of BCR-ABL transcript of 41 patients. The baseline level was 33700 BCR-ABL/β2 microglobuline $\times 10^8$. *Results*. In our investigation we observed a correllation between cytogenetic and molecular results, also a correlation between BCR-ABL transcript levels for peripheral blood and bone marrow. In majority (82 of 105) samples residual disease was detected by quantitative Real-time PCR. Decreasing of BCR-ABL transcript levels less than 2 lg from baseline was associated with greater probability of cytogenetic relapse. 3 lg and more decreasing of BCR-ABL transcript levels from baseline was associated with continious MCR and CCR for all the patients. The patients with cytogenetic relapse had greater median of BCR-ABL transcript level than the patients with MCR and CCR. Cytogenetic relapse was preceded by increasing of BCR-ABL transcript levels. *Conclusions*. For the majority of CML CP patients treated by Glivec it was possible to detect minimal residual disease with the help of quantitative Real-time PCR. Probability of cytogenetic relapse depends upon BCR-ABL transcript level: less than 2 lg decreasing from baseline in our investigation predicted greater probability of cytogenetic relapse. Real-time PCR should be used as routine analyses for CML patients observation as routine analyses of minimal residual disease for CML parients.

1150

ACUTE HEPATITIS AFTER IMATINIB MESYLATE TREATMENT FOR CML: A CASE REPORT AND REVIEW OF THE LITERATURE

P.D. Faccini,[1] D. Corti,[1] P. Del Poggio,[1] E. Pezzica,[1] A. Rosti[1], M. Zucchetti[2]

[1]*Ospedale di Treviglio-Caravaggio, TREVIGLIO, Italy;* [2]*Istituto Mario Negri, MILANO, Italy*

Imatinib is a well tolerated oral anticancer drug. It is mainly metabolised by liver. Severe hepatic dysfunction occurs in fewer than 5% patients treated. We report a case of severe hepatitis documented by liver biopsy and we analyzed similar cases reported in the literature. *Case report.* A 69-year-old female was diagnosed has having CML in the chronic phase. Soon after diagnosis, on 1st August 2003 she started imatinib at a standard dose of 400 mg/d. Three months later she achieved a partial cytogenetic response in the bone marrow and she presented with a slight increase in aspartate aminotransferase (AST 68 U/L) and alanine aminotransferase (ALT 85 U/L). On December 4th imatinib was discontinued because of a progressive increase of hepatic enzymes (AST 158 U/L, ALT 267 U/L). The patient was well and asymptomatic. No other biochemical abnormality was observed. On January 14th, 2004 transaminases peaked at AST 403 U/l and ALT 797 U/l. Serologic tests for hepatitis A, B and C, for EBV, CMV and HSV were all negative. Ultrasonography of the abdomen was normal. Six weeks after imatinib withdrawal we performed a percutaneous liver biopsy. Histological examination revealed a severe necrosis of hepatocytes with some grade of fibrosis and diffuse inflammatory infiltrates.

Table 1. Clinical and laboratory features.

	Our case	*Ohyashiki Leukemia 2002*	*James Leukemia 2003*	*James Leukemia 2003*	*Kikuchi Leukemia & Lymphoma 2004*	*Rocca Gastroenterol Clin Biol 2004*	*Ayoub J Clin Gastroenterol 2005*	*Ikuta Int J Hematol 2005*
Age/Sex	69/F	56/F	58/F	35/F	40/F	64/F	22/F	51/F
Onset of toxicity[$]	12 weeks	11 days	49 ks	22 wks	4 1/2 mos	7 mos	14 mos	3 1/2 mos
Imatinib dose (mg/d)	400	400	250	400	400	200	600	300
Other drugs	yes	no	yes	no	no	yes	yes	?
AST/ALT (U/l)	403/797	220/342	3230/2430	487/159	406/559	20 N/28N	1796/1226	172/167
Rechallenge	yes	no	yes	yes	no	yes	no	yes
Follow-up (Hepatic function)	normalized		AST/ALT at 1,5 N		normalized	normalized	normalized	normalized[&]
Pharmaco-kinetics	metabolite plasma level higher than expected on day 14	not performed	not performed	not performed	significant serum level STI 7 days after stopping drug	not performed	not performed	not performed

$: time after discontinuation of imatinb and biopsy; &: on imatinib treatment with prednisolone and undeoxycholic

On March 2004 bone marrow aspiration documented lost of the cytogenetic response (100% Ph positive metaphases). Histological examination of liver one year after stopping imatinib was greatly ameliorated and showed minimal changes. We decided to reintroduce the drug (100 mg/day). Plasma levels of imatinib were measured by HPLC/MS on day 1, 10 and 14 (baseline, half-an-hour, 1h, 2h, 4h and 8h after administration). The pharmacokinetics profile of imatinib was comparable to that obtained with standard dose in CML and GIST patients but on day 10 at steady state (SS), a marked increase of the main circulating metabo-

lite, N-desmethyl-imatinib was observed. AUCss (ng/ml h) were 10.2 and 6.3 for imatinib and the metabolite, respectively. Imatinib was stopped from day 11 as transaminases increased (AST 84 U/l, ALT 97 U/l). On day 14 the metabolite plasma level was 86 ng/ml, 3 fold higher than the imatinib concentration. *Summary results of the literature.* The other cases of imatinib induced hepatitis reported in the literature are described in Table 1. In all cases there were not any evidence of hepatitis-inducible virus and the liver biopsy showed cytolytic hepatitis of various degree. In one case there was a clear contribution to toxicity of another drug (roxithromycin). Time to onset of hepatic toxicity was quite variable, from a few days to several months of treatment. Interestingly all the patients were females. Transaminases levels usually normalized after interruption of imatinib. All the patients who had had a drug rechallenge had further hepatic dysfunction. In one case it was possibile to continue treatment in association with a corticosteroid. The relatively high level of imatinib N-desmethyl metabolite in plasma of our patient suggests that imatinib metabolism might be involved in the observed hepatic toxicity. Other studies are needed to elucidate this point.

1151

NEW GENOMIC ISSUES ON DER(9) DELETIONS IN CHRONIC MYELOID LEUKEMIA

F. Albano,[1] A. Zagaria,[2] L. Anelli,[2] L. Vicari,[3] V. Liso,[1] G. Specchia,[1] M. Rocchi[4]

[1]*University of Bari, BARI, Italy;* [2]*University of Foggia, FOGGIA, Italy;* [3]*Genetics , A.O.R.N. A. Cardarelli, NAPOLI, Italy;* [4]*DI.GE.MI University of Bari, BARI, Italy*

Background. The Philadelphia (Ph) chromosome is found in more than 90% of chronic myeloid leukemia (CML) patients. Deletions adjacent to the translocation junction on the derivative chromosome 9 were described by several groups. These studies revealed two main points:1) genomic microdeletions were concomitant to the t(9;22) translocation; 2) deleted sequences were located upstream to ABL and downstream to BCR genes. We report a detailed molecular cytogenetic characterization of chromosomal rearrangements in two CML cases bearing deletions on der(9) without the characteristics reported above. *Aims*. We performed a molecular cytogenetic analysis by FISH to precisely characterize chromosomal events occurring in a case bearing a complex variant t(9;22) and in a case with ins(9;22)(q34;q11). *Methods*. Both patients were diagnosed and tested by conventional cytogenetic analysis, fluorescence in situ hybridization (FISH), and RT-PCR. FISH identification of the ABL and BCR genes was performed using a pool of PAC, RP5-835J22 and RP5-1132H12, and the BAC RP11-164N13, respectively. A set of BAC/PAC probes (proximal and distal to ABL and BCR, respectively) belonging to 9 and 22 chromosomes allowed us to define precisely the deletion size. The UCSC database (*University of California Santa Cruz, http://www.genome.ucsc.edu*) was queried for BAC/PAC probe locations and for gene identification. Results. Case #1. FISH experiment with BCR and ABL specific probes revealed one fusion signal on der(22) chromosome, a faint ABL signal on der(9) and a split BCR signal on der(6) and on der(12) chromosomes. Reiterative FISH experiments using appropriate BAC/PAC clones, allowed the precise definition of the complex rearrangement breakpoints. Surprisingly, the detailed molecular cytogenetic characterization of chromosome 9 breakpoint showed genomic loss of about 400 Kb downstream to ABL gene. *NUP214* is the alone gene with known function mapping in the deleted region. According to our FISH results, the revised karyotype was the following 46,XX,t(6;9;12;22)(p22;q34;q13;q11). Case #2. Conventional cytogenetic analysis revealed a normal karyotype. FISH analysis with clones specific for ABL and BCR genes showed a single fusion signal on der(9). These results suggested the occurrence of a cryptic insertion generating a 5'BCR/3'ABL fusion gene on the der(9) instead of 22q11. Further FISH experiments using clones located proximally to BCR showed that a chromosome 22 region of 3 Mb was inserted on 9q34. The use of BAC clones proximal to ABL and distal to BCR showed the loss of chromosomes 9 and 22 sequences on der(9). Two known and one candidate tumor suppressor genes (TSGs) map in the deleted regions: *SMARCB1* (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin subfamily B member 1) and GSTT1 (glutathione S-transferase τ 1) in 22q11, and PRDM12 (PR domain containing 12) in 9q34. Conclusions. Our data indicate that deletions on der(9) in CML cases could also involve chromosome 9 sequences located telomeric to the ABL gene apart from the centromeric sequences previously described. Moreover, genomic microdeletions can be associated to rearrangements involving 9 and 22 chromosomes, such as insertion event, other than reciprocal translocation.

1152

THE SRC KINASE HCK ROLE FOR BCR-ABL INDUCED CELLULAR TRANSFORMATION AND TREATMENT RESISTANCE

A. Lwaleed, M. Lasebai, B. Lwaleed

Southampton University Hospital NHSTrust, SOUTHAMPTON, United Kingdom

Backgrounds. Chronic Myeloid Leukaemia (CML) is a clonal neoplastic disorder of the myeloid precursor induced by the active hybrid tyrosine kinase BCR-Abl. The tyrosine kinase inhibitor STI571 effectively has been shown to control the BCR-Abl positive CML in the chronic phase of the disease. Recent reports have identified the Src Kinase Hck to interact with BCR-Abl kinase possibly through the STAT5 pathways to induce cellular transformation. *Aim*. To investigate the role of Src Kinase Hck on BCR-Abl induced cell transformation as well as its importance in STI571 resistance. *Materials and Methods.* K562 Cells resistant to 5μM of STI571 were cloned and compared to non-STI571 resistant K562 as well as non BCR-Abl expressing cells (KG-1). All cell lines were incubated with (5μM and 10μM STI571) alone or in combination with (5Nm and 10Nm The Src-Kinase inhibitor PP2). Viability studies were conducted using Trypan blue exclusion technique and DNA Fragmentation assays. Protein expression and tyrosine kinase activity were performed using Immunoprecipitaion and Western Blotting techniques. *Results.* The combination of STI571 and PP2 have shown significant synergistic effect in reducing cell viability and tyrosine phosphorylation of both BCR-Abl and Hck in addition to downstream signalling proteins including JAK and STAT. This synergistic effect was observed even at lower concentration of STI571 (5 μM). Significantly, no effect was seen on non-BCR-ABL expressing cells. *Conclusion*. These results further support the importance of the Src Kinase Hck as a downstream signalling kinase for BCR-Abl and the possible role of this pathway in STI571 resistance.

1153

MONITORING OF BLOOD-HISTAMINE LEVELS IN PATIENTS WITH CML DURING TREATMENT WITH IMATINIB

H. Agis,[1] W.R. Sperr,[1] S. Herndlhofer,[1] H. Semper[1], H. Pirc-Donoewenater,[2] O. Haas,[2] C.H. Mannhalter,[1] H. Esterbauer[1], K. Geissler,[3] C.H. Sillaber,[1] U. Jäger,[1] P. Valent[1]

[1]*Medical University of Vienna, VIENNA, Austria;* [2]*St. Anna Children's Hospital, VIENNA, Austria;* [3]*Hospital Lainz, VIENNA, Austria*

Backgrounds. The tyrosine kinase inhibitor imatinib (STI571) is highly effective in the treatment of chronic myeloid leukemia (CML). However, although most patients show a complete cytogenetic response (CCR) to imatinib, drug resistance may occur. Therefore, monitoring of minimal residual disease (MRD) during imatinib-therapy is a pivotal approach. Hence, most MRD parameters currently in use are expensive and require special technology and equipment. In this study, the value of whole blood histamine as a simple MRD marker of CML has been evaluated. *Patients and Methods.* Histamine levels were determined serially in whole blood samples before and during treatment with imatinib in 97 patients with CML by a specific radioimmunoassay. *Results.* Histamine levels were found to be highly upregulated in CML at diagnosis compared to healthy controls, and correlated with the presence of basophils. During treatment with imatinib, blood histamine levels decreased significantly in CML patients and returned to normal levels in those achieving a CCR. In all cases, loss of CCR during therapy was accompanied by an increase in histamine. Whereas the number of basophils were found to correlate well with histamine levels during treatment with imatinib, no correlation was found between histamine and Ph+ metaphases or between histamine and the percentage of BCR/ABL, suggesting that histamine is an independent MRD variable. *Conclusion*. Our data show that whole blood histamine levels are highly upregulated in patients with CML and should be considered as a simple reliable new marker to monitor MRD in patients with CML.

1154

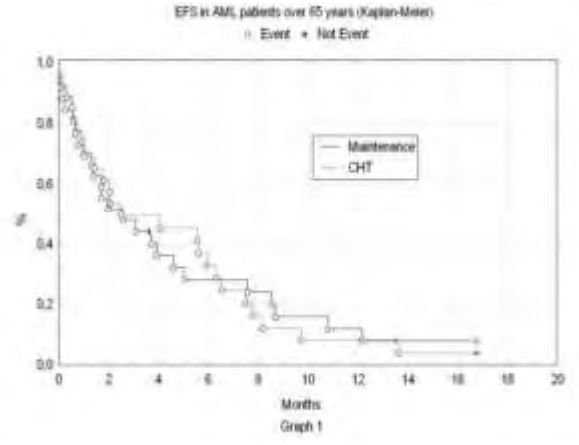
ACUTE MYELOID LEUKEMIA IN THE ELDERLY, INTENSIVE OR MAINTENANCE THERAPY? OUR EXPERIENCE IN PATIENTS OVER 65 YEARS

V. Mettivier,[1] L. Pezzullo,[2] O. Finizio,[2] S. Rocco,[2] L. Bene,[2] G. Nunziata,[2] C. De Rosa[2]

[1]*A.O.R.N. 'A,Cardarelli', NAPLES, Italy;* [2]*Haematology, NAPLES, Italy*

The treatment of acute myeloid leukemia in elderly with age > 65 years is still debated. In literature numerous studies have valued the fea-

sibility of intensive chemotherapy in these patients. The aim of the study is to value the difference in EFS and OS among 2 groups of AML elderly patients treated with intensive chemotherapy (IC) or maintenance (M). From June 2001 to January 2006 we have treated in our Division 54 AML patients, 30 male and 24 female with median age of 73 years (66-90 years). 27 patients (16 M and 11 F with median age of 71 years) have received intensive chemotherapy (I.C. Flag and MICE) and 27 (14 M and 13 F with median age of 78.5 years) have received maintenance (low dose cytarabine and/or support). In IC group 12 patients (45%) have obtained to complete remission (CR) with to EFS and OS media of 4, 47 and 7, 15 months respectively, the rate of TRM has been of 25%. In the M group the CR has been documented in 8 patients (30%) with to EFS and OS media of 4,22 and 4,94 months respectively (graph 1).



This results have shown a best rate of CR in the IC group but the OS and EFS difference is not statistically significant in the two groups (p=0.7). In conclusion the Intensive chemotherapy has not improved the survival in AML elderly patients. New therapeutics strategy is necessary for to improve the EFS and OS in these patients. Interesting is the use of specific monoclonal antibodies (anti CD33) in this poor disease especially in maintenance after a CR obtainable with an intensive or low dose chemotherapy.

1155

ACUTE MYELOID LEUKEMIA IN PATIENTS AGED 70 OR OLDER. EXPERIENCE AT A SINGLE CENTRE

J.N. Rodriguez,[1] E. Martin,[2] G. Rodriguez,[2] M.V. Moreno,[2] J.A. Quesada,[2] A. Palma,[2] J.C. Diéguez,[2] A. Amian,[2] A. Fernández-Jurado[2]

[1]*Hospital 'Juan Ramn Jimnez', HUELVA, Spain;* [2]*Hospital 'Juan Ramn Jimnez', HUELVA, Spain*

The management of old patients with acute myeloid leukemia remains controversial, specially in those cases that can be considered very old patients (aged 70 or older) in which the dilemma therapeutic abstention versus treatment (with low or high intensity) can be considered. We present our experience with this group of patients in the period 1990-2005. During the period of study 56 cases were diagnosed (FAB M3 cases were excluded). Patients were divided into 3 groups according to the treatment: no treatment, low intensity treatment (low doses Ara-C: 10 mg/m^2 s.c. days 1-21) and high intensity treatment (adapted ICE: Idarubicin 10 mg/m^2 days 1 and 3; Ara-C 100 mg/m^2/12h days 1-3; Etoposide 100 mg/m^2 days 1-3). The mean age of patients was 76,29 years (70-85); sex distribution was 29 males and 27 females; mean Karnofsky index was 70,5 (30-100); 39 patients received treatment and 17 did not; overall survival was 5,8 months (median 2; 0,06-90+), almost significant differences were observed in the mean overall survival between the treated and no-treated groups (7,4 vs 2,1 months respectively; p=0,06). In the low intensity group (30 patients) an overall response of 33,3% (6 CR, 4 PR, 13 NR and 7 not evaluable) was observed while in the high intensity one (9 patients) this overall response was 44,4% (4 CR, 0 PR, 3 NR and 2 not evaluable); no statistical differences were observed between both groups (p=0,16). Considering overall survival in these same groups, no statistical differences were observed between them 7,2 (0,25-90+) vs 8,1 (0,5-28+) months (p=0,95) respectively between the low and high intensity groups. Overall survival in the treated group is higher than in the non-treated one, differences almost reach statistical significance (p=0,06). Though no statistical differences have been observed in the overall survival between both groups of treatment, this event could be explained by two reasons: the very long survival in one patient in the low intensity group and the still short follow-up of some patients in the high intensity one. Comparing both arms of treatment, a higher proportion of CR can be observed in the high intensity group (44,4% vs 20%, respectively), however, if this circumstance will contribute to a longer survival is still unknown.

1156

NKG2 RECEPTOR EXPRESSIONS IN UNTREATED ADULT PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA

H.J. Kim,[1] S.Y. Kim,[1] H.Y. Jeong,[1] Y. Choi,[1] W.S. Min,[1] C.C. Kim,[1] T.G. Kim[2]

[1]*CHSCTC,Catholic Univ. of Korea, SEOUL, South-Korea;* [2]*Catholic Hematopoietic Stem Cell Bank, SEOUL, South-Korea*

Backgrounds. Natural killer (NK) cell is one of the important cytotoxic lymphocytes for innate immune response to tumor cells as well as infected cells. The balance of the activating and inhibitory receptors of NK cells can determine the activity of cytotoxicity. Based on recent advances in the understanding of NK cells, baseline expressions of NKG2 might be involved in the immune response that regulate genome integrity in addition to their antigen-specific activation in a challenging clinical condition such as acute myelogenous leukemia (AML). Aims. In this study, we investigated expressions of C-type lectin-like receptors, i.e. *CD94/NKG2A* and *NKG2D*, in adult patients with AML. Together, it is expected that other researchers will also examine the role of ethnic differences in the phenomena described here. *Methods.* PB samples from 24 normal donors and 14 untreated AML patients were enrolled. Flow cytometric analysis using CD56, CD16, CD3, NKG2A, and NKG2D-specific monoclonal antibodies were performed. *Results.* The proportion of CD16+CD3- NK cell and CD56+CD3- NK cell in peripheral lymphocytes were 3.43±3.80% vs 8.26±5.99% and 4.03±3.76% vs 8.61±8.70% in AML and control, respectively. NKG2D+ and CD94/NKG2A$^+$ cells among CD56+CD3- fraction were 35.7±5.4% vs 24.4±3.9% (p=0.13) and 26.8±4.1% vs 37.7±3.7%, respectively. Therefore, *NKG2A* expression of NK cells in AML patients was statistically significantly decreased compare to control (p=0.050). *Summary/Conclusions.* While the expression of *NKG2D*, activating receptor of NK cell is relatively increased, the expression of *CD94/NKG2A*, inhibitory receptor of NK cell is decreased in AML patients, which means some other mechanisms including altered responding ligand(s) are engaged.

1157

INCREASE IN SERUM SOLUBLE HLA-I IN ACUTE MYELOID LEUKEMIAS LEADS TO FAS LIGAND-MEDIATED APOPTOSIS OF CD8+ LYMPHOCYTES

A. Poggi,[1] P. Contini,[2] I. Pierri,[2] A. Albarello,[2] M. Gobbi,[2] M.R. Zocchi[3]

[1]*National Institute for Cancer Research, Genoa, Italy;* [2]*University of Genoa, Genoa, Italy;* [3]*San Raffaele Scientific Institute, Milan, Italy*

Soluble HLA-I (sHLA-I) molecules have been firstly described in the serum and urine of healthy individuals. More recently, it has been shown that the serum level of these soluble molecules is significantly increased in patients with an activation of their immune system, such as during allograft rejection, acute graft versus host disease after bone marrow transplantation, autoimmune diseases or viral infections. Moreover, sHLA-I molecules can be released by tumor cells, and high sHLA-I serum levels have been found in solid cancers, melanomas and lymphomas. Thus, sHLA-I molecule are not specific markers for organ rejection, but rather are affected by inflammatory processes and viral or neoplastic transformation. In this study, we show that high serum levels of soluble HLA class I molecules (sHLA-I, range: 0.7-1.7 mg/mL) and soluble Fas ligand (FasL, range: 0.4-1.9 ng/mL) are detected in patients with acute myeloid leukemia (AML) at diagnosis, compared to healthy donors (sHLA-I range: 0.1-0.6 mg/mL; sFasL range: 0.1-0.4 ng/mL). Both sHLA-I and sFasL serum concentrations increased during chemotherapy. The functional role of sHLA-I molecules either in physiological or in pathological conditions is not clear: it has been described that HLA-I molecules can trigger cytotoxic T lymphocytes to release cytolitic enzymes and pro-inflammatory cytokines. However, we and others reported that sHLA-I molecules bind to CD8 receptors expressed on cytotoxic effector lymphocytes leading to activation-induced apoptosis or cell death mediated by synthesis and secretion of FasL and the consequent inter-

action with Fas expressed by T and NK cells. AML patients' sera were able to induce transcription and secretion of FasL in CD8+ T cells, followed by apoptosis *in vitro*; this apoptosis was inhibited by either anti-HLA-I or anti-FasL specific monoclonal antibodies. These findings closely relate to the *in vivo* up-regulation of FasL transcription observed in peripheral blood lymphocytes from AML patients; in the same cells, mRNA for the antiapoptotic protein Bcl-2 was down-regulated. Interestingly, caspase-8 and caspase-3, both downstream mediators of death receptors-induced apoptosis, were activated *in vivo* in CD8+ cells of AML patients, but not of healthy donors. These data strongly suggest that in AML, increased levels of sHLA-I molecules may be responsible for the elimination of potentially anti-tumor effector cells through a FasL/Fas interaction.

1158

THE NUMBER AND APOPTOSIS OF CIRCULATING ENDOTHELIAL CELLS IN THE PERIPHERAL BLOOD IS SIGNIFICANTLY INCREASED IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND REFRACTORY ANEMIA WITH EXCESS OF BLASTS

A. Wierzbowska, T. Robak, A. Krawczynska, A. Wrzesien-Kus, A. Pluta, B. Cebula, P. Smolewski

Copernicus Memorial Hospital, LODZ, Poland

Objectives. The circulating endothelial cells (CEC) are proposed to be a noninvasive marker of angiogenesis. Material and Methods. We evaluated the absolute counts of CEC, their resting (rCEC) and activated (aCEC) subsets, circulating endothelial progenitor cells (CEPC) as well as apoptotic CEC (CECAnnV$^+$) in peripheral blood (PB) of 70 untreated patients with acute myeloid leukemia (AML) and 23 with myelodysplastic syndrome RAEB type (RAEB). The control group consisted of 30 healthy controls. CEC counts were evaluated by four-colour flow cytometry using a previously described panel of monoclonal antibodies. CEPC were defined as CD45$^-$, CD34$^+$, CD31$^+$ and CD133$^+$. rCEC were defined as CD45$^-$, CD133$^-$, CD31$^+$, CD34$^+$, CD146$^+$ and negative for activation markers (CD105, CD106). CD105 or CD106 positive mature endothelial cells were classified as aCEC. The levels of CEC were correlated with known prognostic factors. Additionally, apoptotic CEC were detected in PB using Annexin V assay (CD146$^+$/Annexin-V$^+$ cells, CECAnnV$^+$). The percentage of CECAnnV$^+$ among the whole CEC number was determined. *Results.* There were highly significant differences in the count of CEC and their particular subtypes between AML and RAEB patients as well as the controls. The results (median counts and ranges) are presented in the Table. The positive correlation between CEC and CEPC counts was observed in both AML (r=0,435; p<0,001) and RAEB (r=0,634; p<0,01). The numbers of apoptotic CEC (CECAnnV+) in both AML and RAEB were significantly higher than in the control (p<0,0001). However, in patients with RAEB the rate of CECAnnV+ was significantly higher than in those with AML (p<0,0001). The number of microvascular origin CEC, depicted by CD36 expression, was also higher in MDS than in both AML (p<0,0001). Moreover, the negative correlation between CEC and absolute counts of white blood cells as well as PB blasts was observed in RAEB but not in AML. Conclusions. The CEC levels are significantly higher in AML and RAEB patients than in healthy subjects. These findings may suggest a relationship between clonal transformation and the substantially increased number of CEC. The rate of CECAnnV+ is significantly elevated in AML and RAEB what may be due to increased turnover of CEC. Distinctly lower propensity of CEC to undergo apoptosis found in AML may correspond with more aggressive clinical course of this disease.

Type of endothelial cells	*AML n=70 (a)*	*MDS-RAEB n=23 (b)*	*Control group n=30 (c)*	*p value*
CEC (/µL)	27,2 (3,9-291,3)	12,5 (4-39,7)	2,95 (0,5-13,1)	a vs. c p<0,0001 b vs. c p<0,0001 a vs. b p<0,0001
a CEC (/µL)	11,35 (0-87,7)	5,4 (1,6-33,1)	0,9 (0-5,2)	a vs. c p<0,0001 b vs. c p<0,0001 a vs. b p<0,01
r CEC (/µL)	11,85 (0-203,6)	7,8 (1,2-21)	1,6 (0,4-10,68)	a vs. c p<0,0001 b vs. c p<0,0001 a vs. b p<0,03
CEPC (/µL)	2,25 (0-40,2)	1,9 (0-12,2)	0,1 (0-1,2)	a vs. c p<0,0001 b vs. c p<0,001 a vs. b p<n.s.

1159

AN ANTECEDENT DIAGNOSIS OF REFRACTORY ANEMIA WITH BLAST EXCESS HAS NO PROGNOSTIC RELEVANCE IN ACUTE MYELOID LEUKEMIA OF THE ELDERLY TREATED WITH AGGRESSIVE CHEMOTHERAPY

F. Ferrara,[1] S. Palmieri,[1] M. Annunziata,[1] C. Copia,[1] F. Pollio[1], P. Correale,[1] G. Mele,[1] C. Califano,[2] A.M. D'Arco[2]

[1]*Cardarelli Hospital, NAPOLI, Italy;* [2]*Umberto I Hospital, NOCERA INFERIORE, Italy*

Background. Host related factors and disease related factors account for the unsatisfactory outcome of acute myeloid leukemia (AML) in the elderly. However, the exclusion in many trials of patients with previously diagnosed myelodysplastic syndrome (MDS) renders uncertain the evaluation of the prognostic relevance of secondary AML (s-AML), defined as AML arising after either a history of chemotherapy or radiotherapy for a previous malignancy or a preceding history of MDS or hematologic malignancies. *Aims*. To evaluate the prognostic relevance of a previous diagnosis of refractory anemia with excess of blasts (RAEB) in terms of complete remission (CR) achievement and duration, survival and feasibility of autologous stem cell transplantation (ASCT) in elderly patients with AML. *Patients and methods*. Among 166 consecutive elderly AML patients observed in the period 2001-2005, 87 cases (median age: 69 years, range 61-81) were enrolled into an aggressive chemotherapy program consisting of a combination of fludarabine and intermediate dose cytarabine given as continuous infusion (c.i.) [fludarabine: loading dose of 10 mg/m^2 at day 0 followed by a c.i. of 20 mg/m^2/24h for 72h; cytarabine: loading dose of 390 mg/m^2 (infusion duration: 3h) 3.5h after fludarabine and then as c.i. at 1440 mg/m^2/24h for a total of 96h]. Fourty-nine patients (56%) were diagnosed as having *de novo* AML, while a diagnosis of s-AML was made in 38 cases (44%). Cytogenetic analysis (76 cases evaluable), showed a normal karyotype in 45 patients (59%), complex karyotype or other unfavorable abnormalities in 31 (41%). No basal characteristic (age, WBC at diagnosis, FAB, cytogenetics) was statistically different between the two groups. A conditioning regimen consisting of 2 days c.i. idarubicin (20 mg/m^2)and 3 days oral busulphan (4 mg/kg) was used. *Results*. Overall, 56 patients (64%) achieved CR, 29 (33%) were able to mobilize a sufficient number of peripheral blood stem cells, and 23 (26%) were actually autografted. CR rate (61% vs 68%, p:0.63), death in induction (20% vs 16%, p:0.78) and primary resistance (18% vs 16%, p=0.97) were no statistically different between the group of *de novo* and s.AML. Median time for neutrophil recovery was similar, while s-AML patients required longer time for platelet recovery (p=0.04). There was no difference as to eligibility for consolidation (87% vs 77%, p:0.54) as well as for mobilization and feasibility of ASCT (16/20 vs 13/18, p=0.85, and 14/16 vs 9/13, p=0.52, respectively). s-AML had negligible impact on overall survival (OS) and disease free survival (DFS), (OS: 8 vs 8 months, p=0.53, DFS: 10 vs 9 months, p=0.41). In the multivariate analysis the only parameter significantly related to either OS or DFS duration was adverse karyotype (p=0.02 and 0.04, respectively). *Conclusions.* A diagnosis of s-AML does not represent a clinically relevant prognostic factor in elderly AML patients treated with aggressive therapy in our series. Furthermore, s-AML patients can be mobilized and autografted with comparable results as opposed to *de novo* cases.

1160

SERUM CONCENTRATION OF SOLUBLE E-CADHERIN (SE-CADHERIN) AND β-CATENIN EXPRESSION IN LEUKEMIC CELLS IN ACUTE MYELOBLASTIC LEUKEMIA (AML) PATIENTS

K. Kapelko-Slowik,[1] E. Sowinska,[1] D. Wolowiec,[1] B. Jazwiec[1], M. Slowik,[2] S. Potoczek,[1] D. Urbaniak_Kujda,[1] L. Ciszak[3]

[1]*Wroclaw Medical University, WROCLAW, Poland;* [2]*Dept.of Ophtalmology, WROCLAW, Poland;* [3]*Institute of Immunology, WROCLAW, Poland*

Background. A tumour transformation is associated with disorders of intracellular structures function, participated in adhesion-dependent signalization, such as: E'cadherin and β-catenin.E-cadherin is a transmembrane glycoprotein that mediates intercellular adhesion. Its soluble form (sE-cadherin) was found in biological fluids of healthy persons. Serum concentration of sE-cadherin are elevated in patients with malignancies of epithelial origin. β-catenin is a multifunctional protein, which plays a role as a component of the cell-cell adhesion apparatus. The function for β-catenin in hematological malignancies has not been reported. Overexpression of β-catenin has been found in Jurcat cells, K562 cell line and HUT-102 cells. Reduction of β-catenin nuclear signalling inhibited proliferation and clonogenicity in these cell lines. The data suggest that ß-catenin can play a significant role in promoting leukemic cell proliferation, adhesion and survival. *AIM*. Aims of our study were: soluble E'cad-

herin (sE-cadherin) serum concentrations comparison in acute myeloblastic leukemia patients (AML) and in controls and estimation of sE'cadherin serum concentration and β-catenin expression in leukemic cells of AML patients at the time of diagnosis. *Patients and methods*. Fourty-eight patients were included: 21 men and 27women aged 20-79years (x=42). According to FAB classification: 1 patient with M0, 10 with M1, 10 with M2, 1 with M3, 15 with M4, 6 with M5, 6 with M6 and 1 patient with bifenotypic leukemia. Sixteen patients reached complete remission (CR). *Results*. We have suggested, that sE'cadherin serum concentration and β-catenin expression in leukemic cells were statistically higher in AML patients with primary resistance to chemotherapy than in AML patients with complete remission (61.9±22.05 vs 57.9±22.6 ng/l, p=0.04 for sE'cadherin and 8.8±.6.2% vs 1.4±1.8%, p=0.00006 for β-catenin expression). We have also indicated the positive correlation between sE'cadherin serum concentration and β-catenin expression in leukemic cells in AML patients and between patients age and sE'cadherin serum concentration. Summary. Our data indicate that sE'cadherin serum concentration and β-catenin expression in leukemic cells could be consider as additional prognostic markers in AML.

1161

A NEW HUMAN ACUTE MONOCYTIC LEUKEMIA CELL LINE TZ-1 WITH T(1;11)(P32;Q23) AND LYMPHOID PHENOTYPES

T.S. Shimizu, M.S. Sagawa, N.A. Awaya, S.O. Okamoto, Y.I. Ikeda, M.K. Kizaki

KEIO University School of Medicine, TOKYO, Japan

Background. Human leukemia cell lines are of great value in investigating basic and applied aspects of cell biology and clinical medicine. Leukemia cell lines have been instrumental in the biological and molecular analysis of recurring chromosome rearrangements, notably translocations. In addition, these cell lines have contributed to a better understanding of the pathogenesis of human leukemias. Translocations targeting the MLL gene at 11q23 have come to represent a paradigm in acute leukemias. It has been reported that there are more than 60 partner genes for MLL. Although the functions of fusion transcripts remain largely unknown, it may be possible that the partner genes are critical for luekemogenesis. There have been 37 leukemia cell lines carrying 11q23 translocation and MLL rearrangements; however, cell lines harboring with t(1;11) (p32;q23) have not been established. *Results*. We report here for the first time a new human acute monocytic leukemia (AMoL) cell line with t(1;11) (p32;q23), designated TZ-1, and herein describe its biological characteristics. TZ-1 cell line was derived from the ascites of a 76-year-old Japanese man with AML M5a and passaged by liquid culture medium for more than a year. EB virus genomic DNA was not detected by quantitative PCR analysis, suggesting that TZ-1 cells are thought to be a continuous cell line. TZ-1 cells revealed typical monocytic features in morphology with folded nuclei, prominent nucleoli, fine reticular chromatin, and abundant blue-gray cytoplasm. The cells were negative for myeloperoxidase, but positive for α-naphtyl butylate esterase stainings. The immunoprofiling as determined by flow cytometry showed that TZ-1 cells are positive for myeloid and monocytic markers (CD13/14/33/34/38/HLA-DR) with B lymphoid markers (CD10/20). Cytogenetic analysis demonstrated a t(1;11) (p32:q23) translocation. FISH using a locus-specific MLL probe showed the gene to be disrupted, the 3' region being translocated on the derivative chromosome 1. A gene located on chromosome 1p32 locus was reported to AF-1; therefore, we next examined to detect MLL-AF-1 fusion transcript by RT-PCR analysis. RT-PCR using both MLL and AF-1 sequence-specific primers was performed on total RNA from TZ-1 cells. RT-PCR analysis demonstrated the MLL-AF1 fusion transcript. Taken together, TZ-1 is a new human acute monocytic leukemia cell line with t(1;11) (p32;q23) translocation and lymphoid phenotypes. *Conclusion*. The established cell line, TZ-1, could provide a valuable model in the study for analyzing the pathogenesis of MLL-AF-1-positive leukemia and developing new agents for this type of leukemia.

1162

CD56 ANTIGEN EXPRESSION IN ACUTE MYELOID LEUKEMIA; CLINICAL AND IMMUNOPHENOTYPICAL IMPLICATIONS

C. Kartsios,[1] M. Papaioannou,[2] M. Ditsa,[2] E. Yiannaki,[2] A. Banti,[2] E. Verrou,[2] K. Zervas,[2] D. Markala[2]

Theageneion Cancer Hospital, THESSALONIKI, Greece; [2]Theageneion Hospital, THESSALONIKI, Greece

Background and Aims. The CD56 antigen expression has been reported in several hematologic malignancies; it is found in 10-30% of cases of acute myeloid leukemia (AML). Its prognostic impact remains uncertain although it has been associated with an unfavorable outcome, especially in AML M2 and M3 subtypes. Recently, two novel $CD56^+$ malignancies (the $CD7^+CD56^+$ myeloid/NK and the $CD4^+CD56^+$ dendritic cell leukemias) were described. We investigated the immunophenotypic identity of the $CD56^+$ AML and its correlations with other disease characteristics. *Patients and Methods.* From 1999 to 2005, 127 samples of fresh bone marrow or peripheral blood of AML patients [M/F: 65/62, median age: 59 (11-85) years], were analyzed in our laboratory. One-, two- or three-color flow cytometry was performed on Coulter Epics cytometer. Positivity for surface (s) antigens expression was set at 20% and for cytoplasmic (c) antigens at 10% of blasts stained with specific monoclonal antibodies for glycophorin A, CD2, sCD3, CD4, CD7, CD8, CD10, sCD13, CD14, CD16, CD19, sCD22, CD33, CD34, CD45, CD56, CD61, CD117, HLA-DR, MPO, lysozyme, lactoferrin, CD79a, cCD3, cCD22, cCD13 and TdT. *Results.* Patients (pts) were classified according to FAB criteria as M0:10 pts, M1/2:51, M3:20, M4:31, M5:12, M6:1 and M7:2 pts. 15.7% of the pts suffered from secondary leukemia. CD56 expression was detected in 29% (38/127) of the pts; this was higher in M1/2 (33%) and M5 subtypes (58%). CD56 positivity was not influenced by age, sex, WBC, blast percentage, Hb and platelet count at diagnosis, LDH, secondary leukemia and extramedullary disease. Statistical analysis revealed a positive correlation between CD56 expression and expression of CD34 (p<0.001, r:0.329), CD2 (p:0.014, r:0.221), CD3 (p:0.047, r:0.180), CD4 (p:0.006, r:0.249) and CD8 (p:0.017, r:0.281) antigens, while a negative correlation was detected for sCD13 (p:0.038, r:-0.187) and cCD13 (p:0.081, r:-0.163) expression. CD56+ AML group expressed also CD33 (87% of the pts), lysozyme (79%), HLA-DR (78%), cCD13 (71.4%), MPO (69%) and CD34 (67%). The comparison between the CD56+ AML group and the CD56- leukemias showed differences in the expression of lysozyme (79% of $CD56^+$ AML vs. 54%, p: 0.044), cCD22 (6% vs. 0%, p: 0.03), CD34 (67% vs. 92%, p: 0.071) and sCD13 (55% vs. 71%, p: 0.066). *Conclusions.* The immunophenotype of $CD56^+$ AML is characterized by the commitment to the myeloid/monocytic lineage (CD33, lysozyme, cCD13, HLADR and MPO). The negative correlation between CD56 expression and sCD13 and cCD13 is a novel finding not previously reported. The further investigation of the possible relationship of the $CD56^+$ AML with the NK/dendritic cell leukemias might be the key to explain its clinical behavior.

1163

DIFFERENTIATION OF HUMAN ACUTE MYELOID LEUKEMIA CELLS IN PRIMARY CULTURE IN RESPONSE TO DERIVATIVES OF METHYL JASMONATE, PLANT STRESS HORMONE

H. Tsumura,[1] H. Kiyota,[2] S. Mishima,[1] H. Ishikura,[1] Y. Honma[1]

[1]*Shimane University Faculty of Medicine, IZUMO, Japan;* [2]*Graduate School of Agricultural Science,, SENDAI, Japan*

Backgrounds. Since several plant hormones and their analogs induce cell cycle arrest and inhibit human cell proliferation, these compounds may be therapeutic agents against human malignancies. Some regulators of plant growth and differentiation have been shown to induce the differentiation of several human myeloid leukemia cells, and might be effective as differentiation inducers to control acute myeloid leukemia (AML) cells. *Methods.* Several myeloid leukemia cell lines were cultured with methyl jasmonate (MJ) and its derivatives. Cell differentiation was determined by nitroblue tetrazolium-reducing activity, morphological changes, α-naphthyl acetate esterase activity and expression of differentiation-associated surface antigens. *Results.* MJ induced both monocytic and granulocytic differentiation of HL-60 cells. MJ activated mitogen-activated protein kinase (MAPK) in the cells before causing myelomonocytic differentiation. MAPK activation was necessary for MJ-induced differentiation, since PD98059, an inhibitor of MAPK kinase, suppressed the differentiation induced by MJ. MJ also induced the differentiation of other human leukemia cell lines. Introduction of a double bond at the 4,5-position greatly enhanced the differentiation-inducing activity of MJ. Although differentiation-inducers potently affect the differentiation

of established cell lines, they may have only modest differentiation-inducing activity in freshly isolated leukemic cells. Therefore, we sought to determine whether the potent derivative of MJ could affect the differentiation of leukemic cells from patients with AML. In the present study, we examined the effect of MJ derivative on the differentiation of AML cells in primary culture and compared this differentiation-inducing activity with those of the well-known inducers all-trans retinoic acid and 1α,25-dihydroxyvitamin D3. Jasmonate derivatives significantly stimulated both functional and morphological differentiation of leukemia cells in some AML cases. This differentiation-inducing activity was more potent than those of all-trans retinoic acid and 1α,25-dihydroxyvitamin D3. Conclusion: Our final goal is perform clinical trials of jasmonate derivatives in differentiation therapy for hematopoietic malignancies, either alone or in combination with other differentiation inducers so that their doses can be reduced. Jasmonate and its analogs are used as flavorings in foods and in cosmetics, and MJ was therapeutically effective in an animal model of lymphoma, suggesting that these compounds may be clinically useful. One novel derivative is a particularly promising therapeutic agent for the treatment of leukemia.

1164

FLAG-(IDA)-MYLOTARG IN THE THERAPY OF RELAPSED AND REFRACTORY ACUTE MYELOID LEUKEMIA. PRELIMINARY RESULTS

F. Fabbiano,[1] R. Felice,[2] S. Magrin,[2] C. Marino,[2] D. Turri,[2] A. Marfia,[2] A. Santoro,[2] S Scim,[2] S. Mirto[2]

[1]*Ospedale 'V. Cervello', PALERMO, Italy;* [2]*Ospedale 'V. Cervello', PALERMO, Italy*

Background. Patients with relapsed and refractory AML have a bad prognosis. In this setting is often difficult to obtain a prolonged complete remission. In the last years Fludarabine and high dose Citarabine with or without Idarubicine and G-CSF have been frequently used (FLAG and FLAG-IDA schedules). The immunotoxin gemtuzumab ozogamicin (Mylotarg)(GO) is a humanized IgG4 monoclonal antibody directed against the CD33 epitope, which is chemically linked to calicheamicin, a highly potent antitumor antibiotic. As a single agent it has been shown to be an effective agent in the treatment of relapsed AML with a tolerable toxicity profile. Recently the United Kingdom Medical Research Council (MRC) published the preliminary results of AML15 trial , which was designed to evaluate the effect of adding GO to each course of intensive induction or consolidation chemotherapy in patients younger than 60 years as first-line treatment. These results are encouraging. *Aims*. We are using this association in the treatment of relapsed and refractory adult AML. *Methods*. Until now 18 patients have been enrolled in this trial and we show our preliminary results. 5 patients were resistant to induction ; 8 patient were relapsed after conventional chemotherapy; 6 of them were in the first relapse, 1 was in the second and 1 in the third; 2 were relapsed after autologous transplant and 3 were relapsed after allogeneic transplantation (1 familiar and 2 unrelated). The median age was 54 years (range 20-74). The median time to the first relapse was 12 months (range 2-96). 16 Pts were treated with Fludarabine 30 mg/m^2 dd 2-6, Citarabine 2,0 gr/m^2 dd 2-6, Idarubicine 10 mg/m^2 dd 4,5,6, Mylotarg 3 mg/m^2 d 1, G-CSF 375 mg dd 1-6. 2 patients with the same schedule without Idarubicine. *Results*. 12 patients obtained Complete Remission (70%), 2 died after therapy and 3 were resistant to this schedule. The last patients is not yet valutable. Out of the 12 patients in CR, 8 are in continuous complete remission (median 6 months , range 2-18). 6 of these underwent allogeneic BMT (2 familiar and 4 unrelated) and 3 are actually in CR, 1 died for progressive leukaemia, 1 relapsed and 1 is not yet valutable. Two are waiting for allogeneic transplant. Two patients relapsed at 3 and 4 months and died for progressive leukaemia. *Conclusions*. FLAG-(IDA)-GO schedule is in our experience a feasible therapy for relapsed and refractory adult acute leukaemia. The toxicity is acceptable. Complete Remission was obtained in 70% of patients with a single cycle. Many of these patients underwent to allogeneic transplant with acceptable toxicity.

1165

FLAG-IDA IN THE TREATMENT OF REFRACTORY/RELAPSED ADULT ACUTE MYELOID LEUKEMIA

D. Pastore, P. Carluccio, A. Liso, S. Sibilla, A.M. Mazzone, G. Spinosa, M. Santodirocco, P. Manduzio, R. Rizzi, G. Specchia, V. Liso

Hematology, BARI, Italy

Background. Although several different chemotherapy combinations have been administered to patients with refractory/relapsed acute myeloid leukemia (AML), the prognosis in this subset of patients is still poor, with a complete remission (CR) rate ranging from 30 to 40%. The goal of reinduction chemotherapy varies from achievement of long-term CR to providing a bridge to hematopoietic stem cell transplantation (HSCT) aimed at prolonging disease-free (DFS) and overall survival (OS). *Aims*. In this study we evaluated the efficacy and the toxicity profiles of the combination of fludarabine, high-dose cytosine arabinoside (AraC), idarubicin and G-CSF in refractory/relapsed AML patients. *Patients and methods.* Between October 1998 and October 2005, 74 AML patients (35 M and 39 F, median age 43 years, range 15-60) were treated with FLAG-IDA (fludarabine 30 mg/m^2, Ara-C 2 gr/m^2 for 5 days, idarubicin 10 mg/m^2 for 3 days and G-CSF 5 mg/Kg from day +6 until neutrophil recovery). All patients underwent cytogenetic evaluation: 4 (5.4%) were in the favourable-risk group, 30 (40.6%) in the intermediate-risk group, 31 (41.8%) in the poor-risk group and in 9 (12.2%) the karyotype was not available. Fifty-four patients (72.9%) were in first relapse: 44 after only chemotherapy, 7 after chemotherapy and autologous peripheral stem cell transplantation and 3 after chemotherapy and allogeneic peripheral stem cell transplantation. Twenty patients (27.1%) were refractory to conventional chemotherapy including cytarabine, etoposide and daunorubicin. *Results.* The overall CR rate was 48.6% (36 of 74): 28 of 54 (51.8%) in relapsed and 8 of 20 (40%) in refractory patients. There were 5/74 deaths (6.7%), 1 due to fungemia (C. tropicalis), 2 to sepsis (*P. aeruginosa*) and 2 to cerebral hemorrhage; 33 of 74 patients (44.5%) were resistant to FLAG-IDA. All patients experienced profound neutropenia (<0.1×10^9/L); in patients achieving remission the median time to reach PMN>0.5×10^9/L and 1×10^9/L was 20 (range: 16-27) and 23 (range: 17-30) days; median time to achieve platelet levels >20×10^9/L and 100×10^9/L was 23 (range:17-28) and 31 days(range 28-38), respectively. During the neutropenic phase, 25 episodes of documented sepsis (33.7%) were observed. Febrile neutropenia lasted a median of 7 days; seven patients had no fever at all. As to non hematological toxicity, the most common side effects were mucositis (60 of 74 or 81.1%) and an increase of serum bilirubin (25 of 74 or 33.7%). After achieving CR, 21 patients received allogeneic stem cell transplantation (11 from a matched donor, 5 from a mismatched donor and 5 from an unrelated donor) and 4 patients received autologous stem cell transplantation; 6 patients were judged unable to receive any further therapy and 5 refused other therapy. In the 36 responders, the disease-free survival (DFS) and overall survival (OS) were 10 (range 4 - 68) and 12 (range 5 - 68) months, respectively; the 21 patients who received allogeneic stem cell transplantation had a DFS of 18 (range 4 - 68) months. *Conclusions*. In our experience, FLAG-IDA is a well-tolerated regimen in refractory/relapsed AML patients; the toxicity is acceptable, enabling most patients to receive further treatment, including transplantation procedures.

1166

NON-ADHERENT LEUKEMIA BLASTS CONVERT INTO ADHERENT FIBROBLASTS WITH THE CHARACTERISTICS OF ORIGINAL LEUKEMIA BLASTS

R. Shirasaki, H. Tashiro, M. Noguchi, N. Shirafuji

Teikyo University School of Medicine, TOKYO, Japan

Background and Aims. The cancer stem cell hypothesis has advanced, and in several malignancies including leukemia cancer stem cells have been identified, which behave similarly to normal stem cells in terms of their self-renewal and self conversion into differentiated cells with regard to their appearance, expression of cell-surface molecules and ability to make an exact recapitulation of the original heterogeneous malignant cells. We estimated whether non-adherent leukemia blast cells converted into adherent fibroblasts with a long-term liquid culture system. *Methods.* From informed patients with acute leukemia or CML, bone marrow cells or the blood were collected. With gradient sedimentation method mononuclear cell-fractions were prepared, which were further cultured for one day to be eliminated of adherent cell-fractions. The non-adherent mononuclear cells were cultured for a long-term, and morphological changes were observed. When fibroblasts were generated, cells were treated with Trypsin, harvested and further cultured. Fibroblasts were divided into subclones, and with RT-PCR analysis the expression of original fusion transcript caused by the chromosomal translocation was estimated. The positive clones were selected, and further characterization including the expression of cell-surface molecules, the capacity of proliferation and the production levels of cytokines were examined. *Result.* When leukemia blasts with fusion product of MLL and ELL, of MYH11 and CBF_ and of BCR and ABL were cultured for a long-term *in vitro*, their morphology changed into fibroblasts, which had similar molecular characteristics to those of original leukemia blasts. The similar expression of cell-surface molecules was also observed to those of original leukemia

blasts. The generated fibroblasts had the same levels of functions to those of normal bone marrow cell-derived fibroblasts. When cultured on leukemia blast-derived fibroblasts, original leukemia blasts proliferated extensively. The generated fibroblasts expressed CD133, which is one of the important markers for cancer stem cells. *Conclusion*. These results indicate that leukemia blasts can create their own environment for proliferation.

1167

PREVALENCE AND PROGNOSTIC SIGNIFICANCE OF FLT3 MUTATIONS IN ACUTE MYELOID LEUKEMIA: ASSOCIATION OF ITDS WITH POOR OUTCOME IN PATIENTS WITH NORMAL CYTOGENETICS

M. Palmisano, E. Ottaviani, T. Grafone, N. Testoni, S. Paolini, M. Rondoni, M. Baccarani, G. Martinelli

University of Bologna, BOLOGNA, Italy

Backgrounds. Acute myeloid leukemia (AML) is a difficult disease to treat, and better treatments are needed. Molecular targeted therapy represents a novel therapeutic approach. Activating mutations of FMS-like tyrosine kinase 3 (FLT3) are present in approximately one third of patients with *de novo* AML and have been implicated in its pathogenesis. The leukemic blasts of most AML patients have the internal tandem duplications (ITDs) in the juxtamembrane region or point mutations in Asp835 and Iso836 codons in the activation loop of the kinase domain (TKD) of the FLT3 receptor. Both mutations result in constitutive FLT3 receptor activity and may play a significant role in leukemogenesis. *Aims.* In this study we have analyzed the incidence and type of FLT3 mutations in a large series of newly diagnosed AML patients. Furthermore, we have evaluated the prognostic impact of FLT3 mutations. *Methods.* The *FLT3/ITD* was determined by polymerase chain reaction (PCR). The mutations of D835 and I836 codons were determined by PCR followed by restriction enzyme digestion (PCR-RFLP). For the estimation of the statistic significance of the differences in the clinical-biological characteristics, between the mutated patients and wild-type patients, it has been used the Student's test t for independent data. The probabilities of overall survival (OS) and disease free survival (DFS) were analysed by Kaplan-Meier method; the differences of OS and DFS, between the mutated patients and wild-type patients, were assessed using the log-rank test. *Results.* Both *FLT3/ITD* and *FLT3/TKD* mutations were found in 15%. Dual mutations were found in 2% of 126 patients. Among the FAB subtypes of AML, the rate of FLT3 aberrations was higher in M4 (27%) and M5 (26%). *FLT3/ITD* was associated to leukocytosis (106.8×10^9/L vs 30×10^9/L in FLT3-wt, p=0.015) and high percentage of circulating blast cells (82% vs 42% in FLT3-wt, p<0.0001). Differently, *FLT3/TKD* mutations were not associated with high white blood cells count and blast cells percentage. *FLT3* mutations were more prevalent in patients with normal karyotype (51%). In this group, DFS and OS were significantly inferior for patients with FLT3/ITD than patients withouth mutations (0 vs 5, p=0.0032; 5 vs 9, p=0.049, respectively). *Conclusions.* We have identified the FLT3/ITD as an independent poor prognostic factor in AML patients with normal cytogenetics. Therefore, targeting FLT3 mutations represents a potential therapeutic target for AML. These results suggest that new treatment modalities, such as therapy with a FLT3 tyrosine kinase inhibitor, are clearly needed for this group of patients with 'standard risk' profile.

Acknowledgments: COFIN 2005 (Myelodisplastic syndromes: pathogenetic models and promise of new therapies),COFIN 2003 (Molecular therapy of leukemias), by FIRB 2001, by the University of Bologna (60%), by the Italian Association for cancer research (A.I.R.C.), by the Italian National Research Council (C.N.R), by Fondazione Del Monte of Bologna e Ravenna (Italy) and A.I.L. grants.

1168

IMPACT OF CHEMOTHERAPY TREATMENT IN AML PATIENTS AGED MORE THAN 60 YEARS OLD. A SINGLE CENTRE EXPERIENCE

M. Arnan, D. Gallardo, C. García, E. Pina, C. Boqué, J.J. Berlanga

Institut Catala d'Oncologia, L'HOSPITALET DE LLOBREGAT, Spain

Introduction: Overall prognosis in elderly patients with acute myeloid leukaemia (AML) is very poor. Nowadays patients older than 60 years who receive conventional chemotherapy achieve complete remission (CR) in <60% and <15% of them are free of disease more than 3 years. Despite palliative treatment strategies has historically been considered dismal, a comparison between aggressive treatment and non-aggressive approach is needed. *Objectives*: We retrospectively analyzed the impact on survival of a conventional treatment with chemotherapy versus a symptomatic approach in AML patients aged more than 60 years old in a single centre. *Patients*. Between January 1974 to September 2005, 409 patients older than 60 years old with a diagnosis of AML were enrolled in our registry. 241 (58.9%) were males and 168 (41.1%) females. The median age of the whole group was 71 years (range 60-93 years). AML subtypes according to FAB classification were: M0 (5.7%), M1 (15.1%), M2 (8.5%), M4 (14.5%), M5 (16.8%), M6 (9.1%), M7 (1.4%) and AML+MDS (28.8%). Survival data were available from 267 patients. According to medical decision, 104 patients (38.9%) received intensive treatment, mainly based on conventional chemotherapy that included an anthracycline with cytarabine. The remaining 163 (61.1%) patients were managed with conservative approaches. *Results.* Analyzing patients aged > 60 years, the actuarial survival at 10 years in the group that received intensive treatment was 11.2% versus 2.2% for those receiving best supportive care (p<0.001). Considering only patients with de-novo AML, actuarial survival at 10 years was also better for patients receiving intensive chemotherapy (14.7% versus 0%; p< 0.001). Patients aged > 70 years (n: 157) also benefit of an aggressive approach (actuarial survival at 10 years: 14.3% vs. 2.7%; p: 0.012), specially when considering patients with de-novo AML (n: 105): 10% vs 0% (p: 0.001). The group of patients aged between 70-80 years (n: 76) with de-novo AML presented an actuarial survival of 10% vs 0% at 10 years (p: 0.005). In contraposition to these results, in patients aged > 60 years with AML and previous MDS no significant differences in survival were observed in basis to receive or not treatment with chemotherapy. *Conclusions.* Our results probe the benefit of treatment in elderly patients with AML mainly in those diagnosed of *de novo* AML.

1169

ADULT ACUTE MYELOID LEUKEMIA (AML) WITH DEL (16) (Q22): A REPORT OF 11 CASES

L.V. Vila,[1] S. Girard,[2] M. Elhamri,[3] X. Thomas[3]

[1]*Hopital Edouard Herriot, LYON, France;* [2]*Laboratory of Hematology, LYON, France;* [3]*Leukemia Unit, LYON, France*

Background. Cytogenetic analysis of leukemic blasts performed at diagnosis is generally recognized as the single most valuable prognostic factor in AML. Three large collaborative studies (MRC, SWOG/ECOG, CALGB) assigned AML patients to one of the three risk groups (favorable, intermediate or adverse) based on pretreatment cytogenetic findings. Cytogenetic hallmarks of core-binding factor (CBF) AML, including t (8;21) and chromosome 16 abnormalities, have been categorized in the favorable group by all three cytogenetic classifications. However, the three cytogenetic risk systems differ with regard to del (16q). SWOG/ECOG classified patients with del (16q) as favorable, while MRC and CALGB did not. *Patients and methods*. We report 11 adults with newly diagnosed AML presenting with del (16) (q22) with the aim of evaluating cytological and clinical features, and the outcome after therapy. Median age was 68 years (range, 33 - 88). The sex ratio male/female was 1.2. *Results*. Median hematological features at presentation were as followed: WBC count at 7×10^9/L (1.5 - 469), hemoglobin level at 94 g/l (71 - 154), platelet count at 85×10^9/L. Circulating blasts were present in 9 cases. Six patients presented with M5 AML subtype of whom 1 patient diplayed eosinophilia, 3 patients presented with M4 AML subtype of whom 1 patient had eosinophilia. Two cases had AML with multilineage dysplasia. Overall 6 cases presented dysgranulopoiesis features, while 4 of them also dysplayed dyserythropoiesis. In all cases karyotype at diagnosis presented numerical and/or structural abnormalities combined with del (16) (q22). Only 3 patients achieved complete remission (CR) (27%) after induction chemotherapy. All three patients relapsed: after 2, 3 and 7 months of CR respectively. Median overall survival was 4 months (0 - 30 months). *Conclusion*. AML patients with true del (16q) are usually found in AML with evidence of myelodysplasia and morphology other than that of acute myelomonocytic leukemia with abnormal eosinophils. They should not be included in the favorable cytogenetic risk group. Consequently, the RT-PCR and/or FISH assays detecting CBFβ-MYH11 gene fusion should be performed in all patients with del (16)(q22) to ensure that they do not harbor a misidentified inv (16) / t (16;16).

1170

FREQUENCIES OF FLT3 ITD AND D835 AS WELL AS JAK2 V617F MUTATIONS IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA

H. Andrikovics, S. Nahajevszky, A. Szilvasi, A. Poros, N. Lovas, B. Kapas, Z. Matrai, S. Lueff, A. Sipos, E. Adam, A. Kozma, E. Laszlo, A. Tordai, T. Masszi

National Medical Centre, Budapest, Hungary

Acquired activating mutations of both fms-like tyrosine kinase 3 (FLT3) and Janus kinase 2 (JAK2) confer proliferative and survival advantage for leukaemic cell clones. Internal tandem duplications (ITD) and Asp835 codon mutations of the FLT3 gene were reported as one of the most frequent genetic alterations in acute myeloid leukaemia (AML). The recently described JAK2 V617F point mutation is supposed to be a causative acquired genetic alteration in chronic myeloproliferative disease (CMPD) that may transform into AML. Data about the frequency of JAK2 V617F point mutation among AML patients are rather limited. In the present study, 132 consecutive adults with newly diagnosed AML (57 males and 75 females) treated in our institute between January 2001 and December 2005 were enrolled. The median age of onset was 49 ± 14 (range 18-83) years. We analysed FLT3 ITD and Asp835 mutations by fluorescent PCR and PCR-RFLP methods; and JAK2 V617F by allele-specific PCR at the time point of diagnosis. ITD was present in 23.5% (31/132) of the patients, Asp835 mutations were detected in 6.8% (9/132). Three patients (2.2%) carried both mutations. JAK2 V617F mutation was positive in 2.2% (3/132) of AML patients. 52% (16/31) of ITD-positive patients had monocytic leukaemia (M5 in the FAB classification system); while only 17% (17/101) ITD negative patients had M5 leukaemia (p=0.02). Regarding the prognostic significance of FLT3 mutations, only patients under 60 years of age receiving curative treatment (n=126; M/F =53/73) were considered. There was no difference in the complete remission rates (CR) between ITD positive and negative patients (76.9% vs. 70.1%), but the relapse rate (RR) was significantly increased in the ITD positive group (65.0% vs. 36.1%; p = 0.023). There was no ITD positive patient in the subgroup with favourable cytogenetic karyotype (n=17). The RR in the FAB-M5 group was not higher than in the whole cohort despite of the high frequency of ITD positivity. There was no difference in CR and RR between the Asp835 positive and negative groups. Only one out of the three AML patients with JAK2 V617 mutation had previous CMPD. None of the V617F positive patients carried the FLT3 mutation. Our results confirm earlier observations on Hungarian patients with AML that FLT3 ITD mutation is a negative prognostic factor. Our data raises the question whether in rare cases, the JAK2 V617F mutation may contribute not only to the development of CMPD, but also to AML.

1171

FLUDARABINE, CYTARABINE (ARA-C), AND IDARUBICIN (FLAI) VERSUS IDARUBICIN, ARA-C AND ETOPOSIDE (ICE) FOR INDUCTION TREATMENT OF YOUNGER, NEWLY DIAGNOSED ACUTE MYELOID LEUKAEMIA PATIENTS: A MULTICENTRE PHASE III TRIAL

G. Martinelli,[1] D. Russo,[2] M. Malagola,[3] A. De Vivo,[4] P.P. Piccaluga,[4] S. Paolini,[4] D. Damiani,[5] A. Candoni,[5] N. Testoni,[4] E. Ottaviani,[4] M. Rondoni,[4] P. Mazza,[5] A. Zaccaria,[5] F. Lauria,[5] P. Avanzini,[5] M. Baccarani[4]

[1]*University of Bologna, BOLOGNA, Italy;* [2]*Chair of Haematology, BRESCIA, Italy;* [3]*Hematology, BRESCIA, Italy;* [4]*Inst. of Hematology 'Sergnoli', BOLOGNA, Italy;* [5]*Division of Haematology, UDINE, Italy*

Fludarabine plus cytarabine (Ara-C) and idarubicin (FLAI) is an effective and well-tolerated induction regimen for the treatment of acute myeloid leukaemia (AML). This phase III trial compared the efficacy and toxicity of FLAI versus idarubicin plus Ara-C and etoposide (ICE) in 112 newly diagnosed AML patients <60 years. Fifty-seven patients received FLAI, as the first induction-remission course, and 55 patients received ICE. Post-induction treatment consisted of high-dose Ara-C (HDAC). After HDAC, patients in complete remission (CR) received a second consolidation course (mitoxantrone, etoposide, Ara-C) and autologous stem cell transplantation (auto-SCT) or allogeneic (allo)-SCT, according to the age, disease risk and donor availability. After a single induction course, CR rate was 74% in the FLAI arm and 51% in the ICE arm (p=0.01), while death during induction was 2% and 9% respectively. Both hematological (p=0.002) and non-haematological (p=0.0001) toxicities, especially gastrointestinal (i.e. nausea, vomiting, mucositis and diarrhea), were significantly lower in FLAI arm. In both arms, relapses were more frequent in patients who were not submitted to allo-SCT. After a median follow-up of 17 months, 30% and 38% of the patients are in continuous CR in FLAI and ICE arm respectively. Our prospective randomised study confirmed the anti-leukaemic effect and the low toxic profile of FLAI as induction treatment for newly diagnosed AML patients.

Funding. supported by COFIN 2003, FIRB 2001, A.I.R.C., C.N.R., Fondazione del Monte di Bologna e Ravenna, European LeukemiaNet founds, A.I.L.grants.

1172

ALLOGENEIC STEM CELL TRANSPLANTATION WITH CD34+ SELECTED GRAFTS IN ACUTE MYELOID LEUKEMIA A LONG-TERM FOLLOW UP

F.C. Campilho, A.C. Campos, S.R. Roncon, C.P.V. Vaz, I.B. Barbosa, R.F. Ferreira, M.M. Mariz, A.C. Carvalhais, P.P. Pimentel

Instituto Portugues Oncologia Porto, PORTO, Portugal

GVHD is a major cause of morbidity and mortality after ASCT. In order to decrease transplant related toxicity, in 1996 we have launched a program of ASCT with *ex vivo* CD34+ selected grafts in AML. Between August 1996 and July 2004, allogeneic stem cell transplantation with CD34+ positive selected PBSC has been performed in 37 patients with acute myeloid leukemia. In 6 pt the cytogenetics was of poor prognosis. In all cases the donors were matched HLA siblings. Median age: 36 years (5 - 55); sex: 15/22 (males/females). Status of disease at transplantation: 1st CR: 32; 2nd CR: 4; early relapse: 1. Myeloablative conditioning regimens used were: busulfan + cyclophoshamide + ATG (BuCy): 26; busulfan + fludarabine (FluBu): 11;. After July 2002 lymphocytes were added back to infuse a target number of 0.3 x 106 CD3+ cells/kg receptor. GVHD prophylaxis was: cyclosporine+methotrexate: 20; cyclosporine only: 17. Median follow-up of surviving patients is 1813 days (range: 560 - 2942). Cells infused×10^6/kg [median (range)]: CD34+: 5.1×10^6 (0.9 - 15.1); CD3+: 0.19 (0 - 0.66). Thirty six evaluable patients engrafted. Five patients had late graft failure (median 354 days, range 113-1204).

Table 1.

	1 year	*3 years*	*5 years*
OS	83.8±6.1%	69.8±7.7%	65.6±8.2%
DFS	70.3±7.5%	61.6±8.1%	53.5±8.9%
RR	20.6±7.0%	31.2±8.5%	31.2±8.5%

There was no significant correlation between graft failure and the number of CD34+ or CD3+ infused. Probability of graft failure at one year was 18,2 + 11,8% after FluBu and 4,6 + 6,6% after BuCy (difference not statistically significant). Acute GVHD grade grade > 2 occurred only in 2 pt. Two of 5 pt with graft failure are alive 3 years after a 2nd graft, in CR and with chronic GVHD. Other 6 patients had chronic GVHD: limited in 3 pt and extensive in 3 pt. Overall survival (OS), disease free survival (DFS) and relapse rate are shown in the table. Overall survival was significantly better if the number of CD3+ cells was < 0.19×10^6/kg; the OS was 94,4 (+5,4)%, 88.9 (+7.4) and 83 (+9)% at 1, 3 and 5 years, respectively, when CD3+ infused < 0.19×10^6/kg and 72.2 (+10.6), 55.6 (+11.7) and 55.6 (+11.7)% when CD3+ cells infused were > 0.19×10^6/kg. There was no significant difference in DFS between subgroups. Transplant related mortality at 100 days and at 1 year was 2,7 (+2.7) and 10.1 (+5.1)% respectively. In conclusion, in our experience ASCT with CD34+ selected grafts in pt wit AML is associated with a low risk of acute and chronic GVHD and seems to reduce transplant related mortality without an increase in relapse risk. These results confirm other studies of ASCT with lymphocyte depleted grafts in AML.

1173

PU.1 AND C/EBPα EXPRESSION IN ACUTE MYELOID LEUKEMIA

A. Di Ruscio, F. D'Alò, F. Guidi, E. Fabiani, G. Leone, M.T. Voso

Catholic University, ROME, Italy

Backgrounds. The myeloid transcription factors C/EBPα and PU.1 play a pivotal role in normal hematopoiesis. PU.1 has been shown to be essential for monocytic development, while C/EBPα is necessary for granulocytic differentiation of hematopoietic precursors. In particular, their reciprocal expression is essential for lineage differentiation. Alterations

of their function are involved in leukaemogenesis and different mechanisms affecting gene transcription, mRNA translation and protein function appear to be important in some AML subsets. *Aims.* We have investigated C/EBPα and PU.1 expression levels, and their reciprocal ratio, in different subsets of AML and correlated these data to morphology, FLT3 ITD mutations and cytogenetics. *Methods.* Bone marrow mononuclear cells (BMMC) were isolated from 117 patients with AML at the time of diagnosis and from 13 normal bone marrows using Ficoll gradient. CD34+ cells were isolated from normal bone marrow by immunomagnetic separation. Granulocytes, monocytes and lymphocytes were isolated from buffy coat of normal donor. AML diagnosis was made according to WHO criteria. Cytogenetic data were available for 73 patients. C/EBPα and PU.1 levels were quantified by real time RT-PCR using 18S as reference gene. *FLT3* ITD mutations were studied using current protocols. *Results.* Heterogeneous expression of PU.1 and C/EBPα was observed in different AML subsets. Higher levels of PU.1 and C/EBPα were observed in promyelocytic and myelomonoblastic leukaemias when compared to normal BMMC (p=0.02 for both). Since most APL patients started ATRA before doing bone marrow aspirate, drug-induced up-regulation of PU.1 could be occurred in these patients' samples as recent reports described. On the other side, lowest PU.1 levels were observed in acute erithroid leukemias and, when compared to normal BMMC, this difference was statistically significant (0.09 vs 4.12, p=0.05). Down-regulation of C/EBPα was observed in AMLs with t(8;21) and acute erithroid leukaemias. We observed that PU.1/C/EBPα ratio was higher in monocytes and decreased progressively from peripheral granulocytes to $CD34^+$ cells. When analysing the distribution of PU.1/C/EBPα ratio, AMLs with t(8;21) showed the highest ratio (median ratio=142), while acute erithroid leukemias had the lowest ratio (median ratio=4.3). In particular, when comparing AML with t(8;21) to all other AMLs and to normal BMMC, the differences in PU.1/C/EBPα ratio reached statistical significance (p<0.0001 and p=0.05, respectively). Since down-regulation of C/EBPα and PU.1 has been described in cell lines expressing FLT3-ITD, we correlated their expression to FLT3 mutations. FLT3-ITD were present in 18 of 112 patients studied (16%) but no differences were observed in PU.1 and C/EBPα levels in mutated and unmutated patients. Moreover, to verify the functional importance of these data, we studied the expression of two C/EBPα and PU.1 target genes, G-CSFR and M-CSFR respectively, in patients with high and low levels of these transcription factors. We found a direct correlation between levels of PU.1 and M-CSFR and between levels of C/EBPα and G-CSFR. *Summary/Conclusions.* C/EBPα and PU.1 expression and alteration of their reciprocal ratio may play a role in the pathogenesis of specific subsets of AMLs. Deregulated expression of these transcription factors may lead to an ineffective transcriptional control of hematopoiesis.

1174

HEART INFARCT AS THE MAJOR CAUSE OF EARLY DEATH OF HEMATOLOGICAL PATIENTS AS IDENTIFIED BY AUTOPSY

A. Carvalhais,[1] F. Kaminski,[2] A. Wawryszuk,[2] A. Waszczuk-Gajda,[2] L. Koperski,[2] A. Wasiutynski[2]

[1]*Instituto Portugus de Oncologia, PORTO, Portugal;* [2]*Medical University of Warsaw, WARSAW, Poland*

While majority of hematological patients die due to either their disease or to adverse reactions to their treatment, there is a paucity of studies that use autopsy to more precisely identify the actual causes of death in each case and to relate this to the clinical diagnosis. More precise knowledge of such causes in hematological malignancies and aplastic anemia would allow to properly focus research efforts and possibly to decrease mortality rates. In this study, the results of 154 autopsies of patients (the largest such series in the literature) with hematological diseases were reviewed and compared with clinical data. They concerned 13.6% of 1129 patients who died in this Department in the years 1996-2005. The most probable causes of death in particular hematological diseases, discordancies between clinical and autopsy diagnoses, and their relation to clinical characteristic were identified in the studied cohort that included primarily patients whose death at this particular time was not explained by the clinical course and in 50% was sudden. Although various infections combined have been found to be responsible for the largest number of deaths (26.6%), the most common single cause was myocardial infarction (29 patients or 18.8%). Moreover, the myocardial infacrtion was found to be the most common cause of death in all age groups (18-42; 43-62;>63 years) and in majority of hematological malignancies (acute myeloblastic leukemia, acute lymphoblastic leukemia, non-Hodgkin's lymphoma, Hodgkin's lymphoma, multiple myeloma, chronic lymphocytic leukemia). Furthermore, this fatal myocardial infarction frequently occurred early after diagnosis and initiation of treatment and without preexisting coronary heart disease. The discordance between clinical and autopsy diagnosis of immediate cause of death was found in 55 patients (35.7%, 95% c.i. 28.2-42.8%) of which 50.9% of cases were considered class I discrepancy according to Goldman's criteria. The myocardial infarction was found to be clinically undiagnosed in 69% of cases. In 41% it was class I discrepant diagnosis. These data suggest that hematological patients require special attention and probably preventive measures concerning myocardial infarction particularly during initiation of antineoplastic therapy.

1175

HUMAN CD34 POSITIVE RESISTANT MYELOID LEUKEMIA CELLS EXPRESS THE EMBRYONIC STEM CELL ANTIGENS: OCT-4 AND CD133

H.T. Hassan, X. Zhai, J.A. Goodacre

Lancashire School Postgraduate Medicine, PRESTON, United Kingdom

In 1942, Globus & Kuklenbeck proposed the presence of embryonic remnants in the sub-ventricular zone of human brain capable of giving rise to malignant tumours [*Arch Pathology, 34, 674-734*]. Recently small population of OCT-4 positive embryonic stem cells has been identified in adult murine bone marrow. Also, human CD133 positive cord blood stem cells co-express OCT-4 and other embroynic genes. The aim of the present study was to investigate the presence of embryonic stem cells markers in human AML CD34 positive cells.

We examined the presence of the two isoforms of human stem cell CD133 antigen and the embyonic OCT-4 antigen in KG1a human CD34 positive resistant AML cells. Both immunofluoresence and immunocytochemical stainings of AML CD34 positive resistant cells with anti-CD133 epitope-1 (clone AC133, Miltenyi Biotec Ltd), anti-CD133 epitope-2 (clone 293C3, Miltenyi Biotec Ltd) and anti-OCT-4 (clone sc-5279, Santa Cruz Biotechnology Inc.) revealed the presence of OCT-4 and CD133 epitope-2 antigens but not CD133 epitope-1 antigen. More than 90% of KG1a AML cells were OCT-4 positive in three experiments using both negative and positive controls. OCT-4 positive cells have significantly larger size than negative cells. The presence of CD133 epitope-2 and not epitope-1 in these AML CD34 positive cells is in line with the CD133 epitope expression in normal endothelial and haematopoietic stem cells. The expression of OCT-4 embryonic antigen in both normal bone marrow and leukaemia cells provide new support for the 60-year old hypothesis of 'embryonic remnants' in adult life being target for and capable of malignant transformation. Further studies are warranted to evaluate the presence of embryonic stem cell antigens in AML blast cells from patients and their functional relevance to resistance to chemotherapy and any prognostic value.

1176

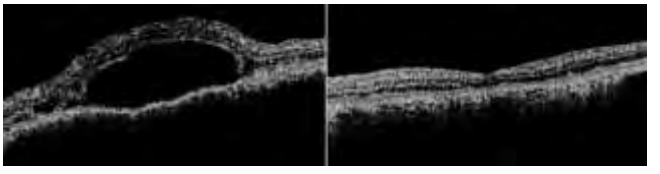
LEUKEMIC INFILTRATION OF THE RETINA AT ONSET OF PHILADELPHIA POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA REVEALED BY STRATUS OPTICAL COHERENCE TOMOGRAPHY (OCT)

A. Candoni, E. Simeone, S. Buttignol, S. Lovato, R. Fanin

University Hospital, UDINE, Italy

A 55-year-old man was admitted with pancytopenia and a loss of vision with a central visual field defect in his left eye. Bone marrow evaluation revealed acute lymphoblastic leukaemia, B-lineage phenotype; cytogenetic and molecular genetic analysis showed t(9;22) and a P210 positivity (BCR-ABL, a2b2). The cerebrospinal fluid was positive for leukaemic cells (100 blast cells/μL). Ophthalmic examination was performed. Fundoscopy showed a lifting and detachment of neuroretinal epithelium in the left eye, which was confirmed by ultrasound examination (vitreous cavity was normal). Stratus Optical Coherence Tomography (OCT) showed a retinal detachment with a choroideal infiltrate in the left eye (Figure 1). Brain and orbital magnetic resonance imaging were normal. The patient underwent induction chemotherapy with daunorubicin and vincristine and intrathecal chemotherapy with

methotrexate, cytarabine and desamethazone. Fundoscopy and OCT, after one course of systemic chemotherapy and two courses of intrathecal chemotherapy, showed complete regression of the retinal infiltration with full recovery of visual function (Figure 2). OCT is a non-invasive way to study the retina that uses reflection of light off the retinal layers to create a high resolution colour tomographic image of retinal structures with an axial resolution of 10 microns or less. In leukaemic patients with a suspicion of posterior ocular segment involvement this technique can be considered as a new and non-invasive diagnostic procedure to see beneath the surface of the retina, permitting detection and follow up of leukemic infiltrates.



1177

TREATMENT RESULTS OF ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN ACCORDING TO ALL-BFM 95 PROTOCOL

M.I. Spasova, A.A. Stoyanova, I.N. Moumdjiev, K.G. Sapunarova

Medical University-Plovdiv, PLOVDIV, Bulgaria

Aims. To evaluate feasibily of treatment of acute lymphoblastic leukemia (ALL) according to the BFM 95 protocol at a single centre in Bulgaria; to assess the 10-years disease-free survival (DFS) in children from all the risk groups; to find factors with prognostic impact on survival from ALL. *Methods.* We studied a cohort of 104 children (59 boys and 45 girls) with ALL, treated at a single centre from January 1996 to January 2006. The mean age of the study group was 6,7 years (from 2 months to 18 years). The chemotherapy and treatment stratification were identical to the ALL-BFM 95 protocol. Patients were stratified into 3 risk groups, based on age, initial white blood cells' count, immunophenotyping, cytogenetics and response to initial treatment: standard-risk (SR), intermediate-risk (IR) and high-risk (HR) groups. Response to initial treatment was assessed by the steroid response on day 8 and hematological remission on day 33 after beginning of chemotherapy. Survival curves were calculated according to the Kaplan-Meier method and statistical significance of differences between curves was determined by the log-rank Mantel-Cox test. Logistic regression was carried out for assessing factors with prognostic impact on survival. *Results.* The patients from the study group were stratified in SR: 31 (29,8%), IR: 52 (50%) and HR: 21 (20,2%) patients. CNS involvement was proven in 2 (1,9%) patients, mediastinal mass - in 14 (13,5%) patients, renal infiltration - in 13 (12,5%) patients. Poor steroid responders were 20 (19,2%) patients and remission was not achieved on day 33 in 6 (5,8%) patients. The 10-years DFS probability was 81,4±1,3% for the SR group, 72,6±0,7% for the IR group and 58,6±1,3% for the HR group (χ^2: 7,1; p=0,027). Independent prognositc factors for DFS, when conducting risk-adapted chemotherapy, proved to be radiologically-proven mediastinal mass (p=0,003; RR: 37, 95% CI: 3,2-416,9) and timely remission induction (p=0,002; RR: 24,6, 95% CI: 2,1-283,9). The majority of relapses occurred within 3 years from diagnosis and most involved the bone marrow. Conclusions. The DFS of the studied group is compatible with the reported from the official BFM study group. Our results on the basis of risk-adapted treatment suggest lack of correlation between survival and the prognostic factors considered previously as significant.

1178

COMPARISON OF HLA CLASS I (A, B, C) AND CLASS II (DRB) POLYMORPHISMS IN IRANIAN PAIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AND CONTROL GROUP

S. Dianat,[1] A. Amirzargar,[2] F. Khosravi,[2] A. Sarafnejad[3]

[1]*Tehran University of Medical Sciences, TEHRAN, Iran;* [2]*Department of Immunology, TEHRAN, Iran;* [3]*Department of Pathobiology, TEHRAN, Iran*

Backgrounds. HLA gene polymorphisms have been extensively studied in various immune-mediated as well as malignant diseases such as leukemia. Since the first study on mouse leukemia by Lilly et al in 1964, the role of MHC molecules as genetic factors affecting the susceptibility or protection against leukemia have been proposed. *Aims.* The aim of this study was to compare the HLA class I (A,B,C) antigens and class II (DRB) alleles frequency between a group of 141 Iranian patients with Acute Lymphoblastic Leukemia (ALL) and two distinct control group of 100 and 180 healthy individuals for HLA class I and II analysis, respectively. Methods. From all of the patients and control subjects blood samples were collected after giving informed consents. HLA class I antigens were determined using serology method while DNA extraction and HLA-DRB typing were performed using PCR-SSP analysis. *Results.* Significant increased frequencies of HLA-A*30: 12.4% vs. 1% (p=0.002, OR=0.074, 95% confidence interval (CI): 0.009-0.58) and HLA-Cw*07: 34.8% vs. 20% (p=0.03, OR=2.15, 95%CI: 1.13-4.08) were noticed in patients with ALL when compared with control group. Patients showed significant lower frequency of some antigens including HLA-B*05 (p<0.0001), B*12 (p=0.005), B*14 (p=0.005), B*41 (p=0.005), B*61 (p<0.0001), B*63 (p=0.0003), B*52 (p=0.0006) and Cw*03 (p<0.0001) than healthy control group. No significant differences were found between patients and control group when compared for HLA-DRB allele frequencies. *Conclusion*: This study suggested the role of some HLA antigens including HLA-A*30 and HLA-Cw*07 as predisposing factors in susceptibility to ALL. While through the antigens with lower frequencies in ALL patients, HLA-B*05, B*61 and Cw*03 showed a stronger and more significant differences. Future studies are needed to confirm these associations in larger samples and investigate the role of specific subtypes using molecular techniques.

1179

MONITORING THE BEGINNINGS AND COURSE OF CENTRAL NERVOUS SYSTEM LEUKEMIA TO THYMIDINE KINASE CONTENT IN CEREBRO-SPINAL FLUID AND BLOOD SERUM IN PATIENTS WITH ACUTE LEUKEMIA

O.A. Kyselova,[1] N.M. Tretyak,[2] N.V. Goryainova,[2] O.V. Myronova[3]

[1]*Institute of Hematology and Transfusion, KYIV, Ukraine;* [2]*Institute of Hematology and Transfusio, KYIV, Ukraine;* [3]*Ukrainian State Medical University, KYIV, Ukraine*

Backgrounds. Thymidine kinase (TK) is an essential enzyme, which is expressed in cell division activity. An increased level of cell division is related to malignant tumour diseases, such as leukaemia and lymphomas. The amount of TK in the blood serum corresponds to the number of dividing malignant cells. Usually, TK activity is not detectable in cerebrospinal fluid (CSF) from healthy human beings, nor is CSF-TK activity detected in most non-neoplastic conditions, but several clinical investigations show that increased levels of CSF-TK at presentation correlate with a high risk of subsequent CNS involvement in patients with responsive acute lymphoblastic leukaemia (ALL). *Aims.* The purpose of the current investigation is determination the prognostic value of TK activity in CSF and blood serum for diagnosis and prognosis of the beginnings and course of central nervous system leukemia (CNS 'leukemia) in acute leukemia (AL) patients. *Methods.* Activity of CSF-TK and blood serum was measured in the different phase of course of AL in 27 patients (ALL- 20 patients, AML- 7 patients) by radioimmunoassay using 5-125 I-iodedeoxy uridine as a substrate. All patients received standard intrathecal prophylaxis of CNS leukemia. *Results.* The first group of the patients (15 persons) consisted of the patients in the different periods of the treatment (during the induction of remission, consolidation and treatment in remission), which have never had any clinical or laboratory features of CNS-leukemia (the duration of observation was 3-24 month). The TK level in CSF was slightly increased at diagnosis (9.8±3 U/l), but became normal after achieving remission. CNS-leukemia in the first acute period of AL was observed in 2 patients and 4 patients had isolated CNS relapse. TK- CSF level in patients with CNS-leukemia at diagnosis was 8.9-15.0 U/l and these patients also had increased level TK in blood serum (22.6 - 46.5 U/l). Those, who had isolated CNS-leukemia relapse, presented TK-CSF level significant higher (from 15.2 till 36.6 U/l). TK level in blood serum in these patients was normal or slightly increased (from 1.4 till 9.0 U/l). *Conclusions.* The results of investigation confirm that TK level in CSF and blood serum appears to be a promising marker in diagnosing and monitoring of CNS-leukaemia involvement in course of the acute leukaemia.

1180

ANOREXIA-CACHEXIA RELATED HORMONES AT DIAGNOSIS AND DURING CHEMOTHERAPY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

M. Moschovi,[1] M. Vounatsou,[2] I. Papassotiriou,[3] G. Chrousos,[4] F. Tzortzatou-Stathopoulou[4]

[1]*Athens University, ATHENS, Greece;* [2]*Blood Transfusion Service, 'Henri Dunan', ATHENS, Greece;* [3]*Biochemistry Dep, 'Aghia Sophia' Hosp, ATHENS, Greece;* [4]*First Pediatric Dep, Athens University, ATHENS, Greece*

Backgrounds. Anorexia and cachexia are common manifestations in children with acute lymphoblastic leukaemia (ALL) at diagnosis. Possible mediators of the anorexia-cachexia syndrome are hormones, cytokines, and adipokines from peripheral tissues, and neurotransmitters, neuropeptides, cytokines, and other hormones in the hypothalamus. Peptide YY (PYY) and ghrelin are gastrointestinal track-derived hormones involved in the short- and long-term regulation of food intake and energy balance. PYY, synthesized mainly by endocrine cells of the terminal ileum and colon, is released into the systemic circulation in response to a meal and participates in signalling the end of the meal at the hypothalamus. PYY exerts its pro-satiety actions possibly through an Y2 receptor-mediated mechanism. Ghrelin, secreted predominantly from X/A-like endocrine cells of the oxyntic glands of the stomach, is primarily secreted in the fasting state, with plasma concentrations falling within one hour of a meal. Its role in food intake and energy balance is opposite to that of PYY, as it exerts orexigenic effects through activation of the hypothalamic neuropeptide Y-Y1 (NPY-Y1) pathway.[1] *Aim.* We evaluated the secretion of PYY and ghrelin at diagnosis and during chemotherapy in children with ALL. *Methods.* Ten patients aged 2-7 years were included in this perspective study. All patients were treated following the same protocol (HOPDA97).[2] A physical examination was performed and blood chemistries were evaluated by standard techniques. Preprandial PYY and active ghrelin levels were determined by specific radioimmunoassays (Linco Research, Inc., USA). Measurements were performed at diagnosis prior to chemotherapy and at several time points prior to each next cycle of chemotherapy for up to 18 months (6-10 measurements per patient). *Results.* Baseline PYY levels were 213.2±85.3 pg/mL, increased significantly to 283.9±72.9pg/mL after the induction and consolidation phase of chemotherapy, and returned progressively to pre-treatment levels at the 6th cycle of the maintenance phase. Baseline active ghrelin concentrations at diagnosis were low (32.6±8.6 pg/mL), fluctuated throughout the study period and stabilized at significantly higher levels (57.4v31.6 pg/mL) after the 8th cycle of maintenance phase of chemotherapy. *Conclusions.* These data suggest that in children with ALL and anorexia-cachexia the levels of PYY decrease with time, as the leukemic burden is eliminated. In contrast, active ghrelin levels are relatively low at diagnosis, remain low during the early cycles of chemotherapy, but normalize with the elimination of the leukemic burden, paralleling the body weight gain trajectory.

References

1. Siahanidou T et al. Clin Chem 2005;51:2131-7.
2. Tzortzatou-Stathopoulou F et al. Eur J Haematol 2005;75:1-9.

1181

SCREENING FOR EVI1 ECTOPIC EXPRESSION IN T-ALL PATIENTS

A. De Weer,[1] B. Poppe,[1] B. Cauwelier,[1] N. Van Roy,[1] N. Dastugue,[2] A. Hagemeijer,[3] A. De Paepe,[1] F. Speleman[1]

[1]*University Hospital of Ghent, GHENT, Belgium;* [2]*Hpital Purpan, TOULOUSE, France;* [3]*University of Leuven, LEUVEN, Belgium*

Backgrounds. Balanced chromosomal rearrangements involving chromosome band 3q26 due to translocations with various partner chromosomes are a recurrent finding in myeloid malignancies. These translocations either contribute to the ectopic expression of, or to the formation of fusion genes involving the EVI1 proto-oncogene. EVI1 transcriptional activation has been reported in up to 10% of acute myeloid leukemias (AML), and is an independent indicator of adverse prognosis. While EVI1 expression is a well documented oncogenic event in myeloid malignancies, EVI1 was presumed not to be involved in lymphoproliferative disorders. However, in an extensive molecular characterization of unselected 3q26 rearrangements, we recently reported the sporadic occurrence of balanced 3q26 aberrations in lymphoid neoplasms. In a t(3;9)(q26;p23) identified in a T-cell Non Hodgkin's lymphoma, FISH confirmed a genomic EVI1 rearrangement. Since these observations suggested a possible involvement of EVI1 in T-cell malignancies, we initiated transcriptional analyses designed to define the presence and frequency of ectopic EVI1 expression in T-ALL. *Aim of the study*. The aim of this study was to investigate the possible ectopic EVI1 expression in T-ALL. *Methods.* We performed real-time quantitative PCR using validated EVI1 primer pairs (1), dedicated to the sensitive detection of ectopic EVI1 expression, on a multi-centre collected series of 87 T-ALL patients and 5 T-ALL cell lines. *Results. EVI1* overexpression was demonstrated to be absent in the 87 patient samples and the 5 tested T-ALL cell lines. *Conclusion*: Although EVI1 overexpression is a poor prognostic marker in AML, it seems not to be involved in the pathogenesis of T-ALL.

References

1. Poppe B et. al. Genes Chromosomes and Cancer 2006;45:349-56.

1182

ECONOMIC AND QUALITY OF LIFE BURDEN OF HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA

W. Feng,[1] T. Tran,[2] M. Stephens,[2] F. Botteman,[2] W. Hay[3]

[1]*Novartis Pharmaceuticals Corporation, FLORHEM PARK, USA;* [2]*Pharmerit, BETHESDA, USA;* [3]*University of Southern California, LOS ANGELES, USA*

Backgrounds. Patients with high-risk acute lymphoblastic leukemia (ALL), including Philadelphia chromosome positive (Ph+) ALL, typically have extremely poor prognosis, experience poor quality of life (QoL) and incur high economic cost. *Aims.* This study examined the economic and QoL outcomes for high-risk ALL patients including Ph+ ALL. *Methods.* A systematic search of the English-language literature published between 1990 and 2005 was conducted. Additional searches were conducted from the retrieved article bibliographies and appropriate conference proceedings (2000-2005). Articles selected for inclusion were prospective or retrospective studies specifically designed to examine burden of illness, direct medical costs, cost drivers, or QoL outcomes of ALL and treatments. *Results.* Of 798 abstracts screened, 106 met selection criteria and were reviewed in detail. Forty-nine and 47 studies focused on the economic and QoL aspects of ALL, respectively. Patients with high-risk ALL are usually defined by cytogenetic alterations (e.g., t(9;22)(q34;q11), t(4;11)(q21;q23)), age, increased white blood cell count, and slow response to therapy. The average annual direct medical cost per high-risk ALL patient ranged from $100,000 to $136,000 as compared to $40,000 to $74,000 for a standard-risk ALL patient. Hospitalization was the major cost component comprising 50%-80% of total direct costs. Major hospital cost drivers included infections, chemotherapy, growth factors, transfusions, and transplantation. These drivers resulted in more frequent hospitalizations and longer ICU lengths of stay for high risk patients. High-risk ALL patients typically had psychological problems and physical complaints, especially in domains of emotion, cognition, and pain. Furthermore, high-risk patients were more likely to have poorer QoL than standard-risk patients due to higher relapse rates and increased need for transplantation. *Conclusions.* ALL exacts a substantial economic and QoL burden on patients, their loved ones and society in general. This burden appears particularly heavy for high-risk patients, such as patients with Ph+ ALL, one of the worst prognosis in ALL. Imatinib either as a single agent or as part of combination regimens has been reported to extend disease-free-survival and improved quality of life among patients with Ph+ ALL in clinical studies (Pui et al. NEJM 2006). Further research is undertaken to evaluate the economic and QoL benefits of imatinib as compared to the current therapies in the treatment of Ph+ ALL.

1183

TREATMENT OF RELAPSED PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA AFTER MYELOABLATIVE STEM CELL TRANSPLANTATION WITH IMATINIB

B. Piatkowska-Jakubas, D. Hawrylecka, P. Mensah-Glanowska, A.B. Skotnicki

Jagiellonian University, KRAKOW, Poland

Backgrounds. Philadelphia chromosome-positive acute lymphoblastic leukemia have a markedly poor prognosis when treated with conventional chemotherapy alone. Even with intensive treatment such as allogeneic transplant, a large proportion of patients relapse. *Aims and Methods.* We described here two patients with Ph+ ALL who relapsed after HLA-identical sibling donor stem cell transplantation and were treated

with shortened course of reinduction chemotherapy and imatinib mesylate (400 mg/day). *Results.* One of these (male age 36) received imatinib for MRD positivity detected in PCR after SCT. Bcr-abl transcript became undetectable after 1,5 month of imatinib treatment. STI and immunosuppressive therapy was discontinued at day +120. These response was not sustained and the patient relapsed 9 month after alloSCT despite of chronic GVHD symptoms. He was induced into hematological remission with imatinib combined with additional mild chemotherapy and achieved complete donor chimerism with PCR negativity. Because of extensive chronic GVHD (skin, liver, oral sicca, ocular sicca, bronchiolitis obliterans without thrombocytopenia) systemic steroids therapy was introduced and imatinib (400mg/day) was simultaneously continued. At present, the patient has a 19-month post-transplantation follow-up and is in stable molecular remission as evaluated by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) for the BCR/ABL fusion gene transcript, cGVHD has partially resolved and patient was able to reduce immunosuppression. In the notably during imatinib treatment we have observed unusual clinical improvement of bronchiolitis obliterans (BO) process confirmed by computed tomography and pulmonary function testing. Another patient relapsed 6 month after alloSCT and obtained complete hematological remission with 100% donor chimerism and PCR negativity after mild chemotherapy followed by imatinib. Imatinib was well tolerated and did not induce GVHD. At 13 month follow-up patient is still in complete hematological and molecular remission. DLI is planned. *Conclusions.* Imatinib combined with low dose chemotherapy is a promising therapy option in Ph+ALL patients relapsed after alloSCT not eligible for intensive treatment, achieving remission prior to DLI and maintain remission during immunosuppressive therapy of GVHD. Further studies to elucidate a role of imatinib (VEGF gene transfer and platelet-derived growth factor receptor inhibitor) in transplant BO are needed.

1184

BURKITT-LIKE LYMPHOMA: A NEW TREATMENT PROTOCOL BLL-M-04

A. der Baryakh, S. Kravchenko, M. Kremenetskaya, T. Valiev, E. Zvonkov, N. Obukhova, I. Vorobjev

Russian Hematological Research Center, MOSCOW, Russian Federation

Backgrounds. Burkitt-like lymphoma (BlL) is a clinical and morphological variant of Burkitt lymphoma. It is the most aggressive B-cell lymphoid neoplasm, which proliferative activity approximates 100%. At the same time BlL is one of the most chemosensitive lymphoma. Intensive chemotherapy allows achieving remissions in 70-90% cases and increasing a common 5-year survival up to 60-75% (according to the stage of disease). *Aim*: to evaluate an efficacy and toxicity of protocol BlL-M-04. *Methods.* Seventeen patients (12 males and 5 females, mean age 25 years) were eligible for inclusion in the study if they had a diagnosis of Burkitt-like Lymphoma (BlL). All the patients participated in the study performed in Russian Hematological Research Center between January 2002 and December 2005. Fourteen patients were not treated previously. Three patients from another clinics had a diagnosis of Diffuse B-large cell Lymphoma and were treated with CHOP (cyclophosphamide, vincristine, adriamicine and prednisolone), but in Russian Hematological Research Center were used additional diagnostic methods (fluorescent in situ hybridisation, flow cytometry, immunohystochemistry) and BlL was diagnosed. The BlL staging criteria developed by S. B. Murphy was used to stage the patients. The stage I, II, III, IV was diagnosed in 2, 1, 6, 8 patients respectively. Bone marrow was involved in 5 patients, neuroleukemia - in 5 patients. B-symptoms (night sweets, fever, weight loss) were in 13 patients. Serum lactate dehydrogenase level was increased in 15 patients. The main aim of a new treatment regimen became an intensification and treatment duration reduction in patients with BlL. Our new treatment protocol basis on standard NHL-BFM-90 protocol. We know that BlL is a chemosensitive tumor and regresses after 1-2 courses of chemotherapy. Despite the initial tumor mass we decided to treat BlL according to 4 courses (2 inductional and 2 consolidational). According to the fact that BlL is the most sensitive to high dosed methotrexate and cytarabine we used this drugs in the induction phase to achieve the most cytoreductive effect. Courses A and C were used for remission achievement. Doxorubicine was added to course A, methotrexate - to course C. Consolidative courses were the same as inductional courses. So, we used A and C courses (without B), intensifired with course B drugs (doxorubicine and methotrexate), interval between courses was 21 day. *Results.* seventeen patients were treated with BlL-M-04 protocol, 16 patients (94%) achieved a CR after 1-2 courses (9 patients - after 1st course, 7 - after 2nd). Fifteen patients are in a first CR during 16,5 months (median 5-27 months). Two patients were died. The course of death was traumatic subdural hematoma in patient with chemotherapy induced thrombocytopenia up to 40×10^9/L in CR. The 2d patient dead because of severe fungal sepsis during remission induction. Treatment duration was 3,0-3,5 months. Myelotoxic agranulocytosis completed all courses. The most number of infectious and hemorragic complications of treatment were registrated during the first course A, that can be explained initial severe patient condition and stage IV in the most patients. An unfavorable prognostic factors, which increase the number of chemotherapy complications are stage IV, acute renal failure, inadequate previous treatment (surgery and chemotherapy). *Conclusions.* BlL-M-04 is a high effective protocol - 94% of patients achieved a remission, 88% are alive in CR, which was detected after 1-2 courses. No one patient was resistant. We conclude, that the most number of relapses are after 8-12 months of treatment and after 24 months we can think about full recovery. So, in the nearest future we can evaluate an efficacy of a new treatment protocol BlL-M-04. The usage of this protocol can achieve a rapid BlL regression and treatment duration reduction because of treatment intensification and acceptable toxicity.

1185

BURKITT'S LYMPHOMA IN KOREA: CLINICAL MANIFESTATIONS AND EFFICACY OF MODIFIED CALGB 9251 REGIMEN (BNHL)

C. Suh, D. Lee, S. Kim, Y.H. Cho, J. Huh

Asan Medical Center, SEOUL, South-Korea

Backgrounds. Clinical manifestations of sporadic Burkitt's lymphoma of Asia including Korea has not been well informed. When Korean patients with Burkitt's lymphoma were treated following the protocol proposed by the Cancer and Leukemia Group B (CALGB) 9251, grade 4 mucositis was reported in every patient. Thus, we had modified CALGB 9251 protocol and named BNHL. *Aims.* This study was aimed to show the clinical manifestations and to evaluate efficacy and toxicity of BNHL in patients with Burkitt's lymphoma in a Korean single center. *Methods.* Between January 1998 and July 2005, 25 patients who were diagnosed as Burkitt's lymphoma at Asan Medical Center were available to evaluate the clinical features. Among 25 patients, 12 patients treated with BNHL were available. In BNHL protocol, we reduced the dose of methotrexate (1,500→1,000 mg/m^2/day on day 1 of cycles 1, 3, and 5) and etoposide (80→50 mg/m^2/day on days 4 and 5 of cycles 2, 4, and 6. *Results.* Median age was 50.4 years (range: 17-77) and 15 patients were male. Among 25 Burkitt's lymphoma, 20 patients had extranodal involvement, and 9 patients had 2 or more extranodal involvements. Extranodal sites were bone marrow, GI tract, genitourinary organ, bone, and lung, in decreasing order. Among total patients, 16 were in stage III or IV, and 9 were in stage I or II. Twelve patients had B symptoms, and 16 patients had high or high intermediate score of age adjusted international prognostic index (IPI). For 12 patients treated with BNHL, median follow-up duration was 13 months (range: 3-20 months). Among patients treated with BNHL, 9 patients achieved CR, and 3 patients achieved PR. The event free survival (EFS) rate at 1 year was 54% (95% CI, 39-69%) and median EFS was not reached. Among 9 patients who achieved CR, 2 patients were relapsed. Three patients died as a result of treatment-related complications (2 patients) or progressive disease (1 patient). Median overall survival (OS) was not reached yet, and 1-year OS rate was 64% (95% CI, 50-78%). All patients treated with BNHL had hematologic toxicities of grade 3 or 4 neutropenia/thrombocytopenia. Grade 3 or 4 non-hematologic toxicities were; mucositis (67%), infection (58%), hepatic toxicity (42%), peripheral neuropathy (25%), and azotemia (25%). *Summary/Conclusions.* Korean patients with Burkitt's lymphoma had worse age-adjusted IPI score than those in western countries. Extranodal involvements were more frequent. Despite these poor risk factors, BNHL modified from CALGB 9251 showed satisfactory efficacy and acceptable toxicities in Korean Burkitt's lymphoma.

1186

ACUTE PERIPHERAL NEUROPATHY FOLLOWING HYPERCVAD REGIMEN FOR MANTLE-CELL LYMPHOMA

M. Perez-Encinas

Hospital Clnico Santiago de Compostela, SANTIAGO DE COMPOSTELA, Spain

Backgrounds. HyperCVAD is an effective regimen for the mantle cell lymphoma (MCL) with potential cerebellar toxicity, but non acute peripheral neuropathy like Guillain-Barre syndrome has been reported. *Aims.* We report three patients with MCL that presented a severe acute polyneuropathy probably secondary to the hyperCVAD/MTX-AraC reg-

imen. *Methods.* retrospective study of MCL patients treated in our Hospital with the hyperCVAD regimen and review of the literature. *Results*. Case 1: 54 years old male with a history of polyarteritis nodosa and cerebral infarction, diagnosed in 1996 of MCL. Four weeks after the second course (MTX-AraC) the patient presented with proximal pain and weakness in arms, than in hours progressed to the legs, and in two days to a quadriplegia and inability to swallow. An electromyography (EMG) showed a motor demyelinating polineuropathy. An MRI and CSF study didn't showed neurological infiltration. The patient was treated with e.v. immunoglobulin with a partial recover of strength. Six months later the lymphoma relapsed and the patient died. Case 2. A 58 years old male with a history of chronic renal failure, an incipient polyneuropathy and two cadaveric renal transplantations, that was diagnosed of MCL in 1998. Three weeks after the four hyperCVAD courses (the MTX-AraC was omitted) the patient presented a progressive and severe weakness in legs and pain in proximal muscles of limbs, and four days later the patients need to bed resting. An EMG showed a motor and sensorial axonal polineuropathy. An MRI and CSF study didn't showed neurological infiltration, and the lymphoma was in complete response. There was non response to e.v. immunoglobulin therapy and the patient died by a sepsis. Case 3. A 53 years old man with a history of diabetes mellitus diagnosed in 2005 of MCL and treated with Rituximab-hyperCVAD/MTX-AraC. Three weeks after 5 course (3 hyperCVAD and 2 MTX-AraC) the patient started with peribuccal paresthesias and pain in dorsal muscles. A week later the patient presented with ptosis, a more severe muscular pain, a depressed tendon reflexes and a progressive weakness in the extremities, more severe in the upper extremities. An EMG showed a motor and sensorial axonal polineuropathy. An MRI and CSF study didn't showed neurological infiltration. The patient was treated with e.v. immunoglobulin, metilprednisolone and plasmapheresis with only a temporal improvement. After one month the pain ceased but the neuropathy progressed with a complete quadriplegia, bladder atony, and a deficient vibration and positional sensory.Four months later the lymphoma relapsed and the patient died. *Conclusion*. The hyperCVAD is an intensive regimen with significant toxicities and we think that acute peripheral neuropathy was a toxic manifestation. A previous peripheral neuropathy or risk factor as uremia and diabetes mellitus could predispose our patients to developing a severe polyneurotopathy. These features must to take into account before the choice of hyperCVAD regimen.

1187

PHASE II CLINICAL EXPERIENCE WITH BORTEZOMIB IN PATIENTS WITH INDOLENT NON-HODGKINS LYMPHOMA AND MANTLE CELL LYMPHOMA

V.P. Pitini,[1] G.A. Altavilla,[1] C.A. Arrigo,[1] C.N. Naro,[1] V.C. Cavallari,[2] M.R. Righi[3]

[1]*Medical Oncology, MESSINA, Italy;* [2]*Ultrastructural Pathology, MESSINA, Italy;* [3]*Human pathology, MESSINA, Italy*

Backgrounds. Bortezomib is a novel small molecule, which is a potent selective and reversible inhibitor of the proteasome. Bortezomib has also shown activity *in vitro* against a variety of lymphoma cell lines including mantle-cell lymphoma (MCL)-derived and diffuse large-cell lymphoma-derived cell lines. *Aims.* The preclinical results and clinical observations provided the rationale for this phase II trial of Bortezomib for the treatment of patients with relapsed or refractory B-cell non Hodgkin lymphoma. *Methods.* To date, we have treated 11 previously treated patients (pts.), (8 males and 3 females with a median age of 60 years, median number of prior therapies 4) with relapsed or refractory indolent lymphomas including: 2 pts. with small lymphocytic lymphoma; 2 pts. with follicular lymphoma; 7 pts. with MCL. Patients were treated at a dose of 1.5 mg/m^2 twice weekly for two consecutive weeks with a one-week rest period. *Results.* No grade III or IV toxicities were observed, save one patient that developed a grade 3 sensory and motor neuropathy. Re-staging studies were routinely performed after two complete cycles of therapy. All pts. with small lymphocytic lymphoma and follicular experienced PD. Among the seven assessable pts. with MCL there was two pts. with CR, two pts. with PR, one patient with SD and two pts. with PD. In responders pts., the median time to progression was not reached with a median follow-up of 9.1 months. *Conclusions.* These data continue to support the biological activity of Bortezomib in pts. with select sub-types of indolent non-Hodgkin's Lymphoma.

1188

LIPOSOMAL DOXORUBICIN IN THE TREATMENT OF LYMPHOMA PATIENTS

L. Rigacci, L. Nassi, R. Alterini, V. Carrai, F. Bernardi, A. Bosi

Azienda Ospedaliera Universitaria, FLORENCE, Italy

Backgrounds. Myocet (liposomal doxorubicin) has an improved pharmacokinetic profile with less myelosuppression and GI toxicity and has a reduced risk of cardiotoxicity at dose level equivalent to standard formulations of doxorubicin. *Methods.* From june 2003 we replaced the conventional doxorubicin with liposomal doxorubicin (Myocet 50 mg/m^2 in COMP and 25 mg/m^2 in MBVD) for the treatment of 25 patients (pts). They were selected pts: elderly pts, pts with impaired cardiac function, pts previously treated with doxorubicin. Twenty pts with NHL were treated with R-COMP and 5 Hodgkin's lymphoma with MBVD. *Results.* The median age was 68 years (range 54-76). Three pts were stage I, 7 stage II, 6 stage III and 9 stage IV. According to histology: 16 were DLBL, 3 mantle cell lymphoam and one marginal zone lymphoma. According to IPI score, for NHL only, 7 pts were low risk, 6 low-intermediate, 6 intermediate-high and 1 high risk. Seven were pretreated with doxorubicin (490 mg median cumulative dose), 7 pts showed impaired cardiac function (4 ischemic, 7 hypertensive and 2 hypokinetic). The median left ventricular ejection fraction (LVEF) at diagnosis was 59% (range 45%-70%). We performed cardiac evaluation at diagnosis, after three cycles and at the end of therapy. All pts but one had no change in LVEF, one patient (4%) presented a myocardial disfunction resolved with medical therapy. The average dose of liposomal doxorubicin for patients who concluded thearpy was 465 mg (range 80-600 mg). At the moment 21 out 25 patients are evaluable for response: 15 pts obtained a complete remission (71%) three a partial remission with an overall response of 86%, one patient stopped therapy due to myocardial disfunction and two patients died one for a stroke and the other for gastrointestinal bleeding. After 130 cycles we have observed one toxic event and two concomitant complications. No significant hematological toxicity was recorded. Three pts died of disease and after a median observation period of 12 months (range 1-32) the overall survival was 80%. *Conclusions.* We conclude that liposomal doxorubicin allows to treat patients with concomitant diseases which could limit the use of conventional anthracycline. Myocet is feasible and effective in a subset of patients with very negative characteristics at diagnosis. It reduces cardiotoxicity risk without reducing chemotherapeutic efficacy.

1189

INTERIM ANALYSIS OF MULTICENTER PHASE II TRIAL OF GEMCITABINE, ETOPOSIDE, CISPLATIN AND DEXAMETHASONE (GEPD) CHEMOTHERAPY IN PATIENTS WITH PRIMARY REFRACTORY OR RELAPSED NON-HODGKINS LYMPHOMA (NHL)

W.S. Lee,[1] K.H. Kim,[1] Y.D. Joo,[1] H.J. Shin,[2] J.S. Chung,[2] G.J. Cho,[2] H. Kim,[3] Y.J. Min,[3] J.H. Park,[3] Y.S. Kim,[4] C.H. Sohn[1]

[1]*Inje University Busan Paik Hospital, BUSAN, South-Korea;* [2]*Pusan National University Hospital, PUSAN, South-Korea;* [3]*Ulsan University Hospital, ULSAN, South-Korea;* [4]*Kosin University Gospel Hospital, BUSAN, South-Korea*

Backgrounds. Platinum and etoposide-based chemotherapy has been used extensively as salvage therapy for NHL. Gemcitabine was studied in a number of phase II trial as a single agent against relapsed NHL. In these studies, the single-agent gemcitabine as salvage treatment showed moderate activity and mild toxicities against the heavily pretreated lymphoma patients. Aims. We access the efficacy and safety of GEPD chemotherapy (gemcitabine 700 mg/m^2 continuous i.v. on day 1, 8; etoposide 40 mg/m^2 i.v. on days 1-4; cisplatin 60 mg/m^2 i.v. on day 1; dexamethasone 40 mg i.v. on days 1-4) in relapsed or refractory NHL patients. Courses were repeated every 21 days. Methods. Patients with histologically proven diagnosed NHL, documented relapse or resistant disease were eligible. All patients received GEPD chemotherapy as salvage treatment The primary end point was a response rate after 2 cycles. Patients could then proceed to stem cell collection using mobilizing regimens (ESHAP or GEPD plus filgastrim) and followed by autologous stem cell transplantation or continued to additional 4 cycles of GEPD. Results. Between Jan 2005 and Dec 2005, 15 patients (8 males and 7 females) were enrolled in the study. Median age was 55 (range 16-75) years. Of these patients, 7 patients (46.7%) had diffuse large B-cell lymphomas. Median follow-up duration was 5.1 (range 1.0-13.0) months. After 2 cycles, there were 2 (13.3%) complete response and 5 (33.3%) partial response. There was an overall response rate of 46.7%. Myelosuppression was the dose-limiting toxicity. 11 patients (73.3%) experi-

enced grade 4 neutropenia and 6 patients (40.0%) experienced grade 4 thrombocytopenia. Autologous stem cell collection was attempted in the 7 patients and was successful in all cases. The median number of CD34-positive cells collected was 5.2 (range, 2.8-11.6)×10^6/kg. Of 13 patients < 66 years, 4 patients (30.8%) proceeded to stem cell transplantation. Conclusions. GEPD chemotherapy in patients with primary progressive or relapsed NHL is effective as salvage therapy and does not interfere with the ability to harvest autologous stem cells for subsequent transplantation. A final analysis is planned after total 40 patients are enrolled.

1190

R-FND VS R-CHOP TREATMENT AS FIRST LINE THERAPY FOR FOLLICULAR LYMPHOMAS: A SINGLE INSTITUTIONAL EXPERIENCE

G. Kadikoylu,[1] A. La Sala,[2] P.R. Scalzulli,[2] L. Melillo,[2] A. Falcone,[2] P. Musto,[2] N. Cascavilla[2]

[1]*Adnan Menderes University, AYDIN, Turkey;* [2]*'Casa Sollievo della Sofferenza' Hosp., SAN GIOVANNI ROTONDO, Italy*

Background. High response rates in follicular lymphoma (FL) with the *FMD* protocol have been previously reported. The monoclonal anti-CD20 antibody Rituximab has been shown to induce a high response rate in FL patients and to improve outcome when associated with classic regimes (CVP or CHOP). *Aims.* We have evaluated the impact of R-FMD as compared to R-CHOP as a first line therapy in patients with follicular lymphomas, in terms of: complete response (CR), overall survival (OS), toxicity and the efficacy of PCR molecular analysis in predicting clinical and molecular remission. *Methods*. Between September 2002 and April 2005, 28 pts with FL were enrolled in the study. Fourteen patients (M/F: 8/6, median age 54 years) received R-FMD treatment in stage II-IV, FLIPI score: intermediate grade 3 pts, high grade 11 pts. R-FMD regimen was administered every 28 days for six cycles: Fludarabine 30 mg/m^2 e.v. days 1-3, Mitoxantrone 10 mg/m^2 day 1, Dexamethasone 20 mg days 1-3 and Rituximab 375 mg/m^2 day 1. PCR molecular analysis was performed in 12 patients at diagnosis, showing in 10 (84%) of them bcl-2 rearrangement. Fourteen patients (M/F: 7/7, median age 56 years) received R-CHOP treatment in stage II-IV, FLIPI score: intermediate grade 4 pts, high grade 10 pts. R-CHOP regimen was delivered every 21 days for six cycles, preceded on day 1 by Rituximab 375 mg/m^2. PCR molecular analysis was performed in 10 patients at diagnosis showing in 9 (90%) of them bcl-2 rearrangement. *Results*. Arm R-FMD: An overall response (13 (93%) CR and 1 (7%) partial response-PR) was achieved in all patients; the pts in PR achieved CR after Zevalin. Actually, after a median follow up of 28 months, all 14 pts resulted in CR. At the end of treatment, bcl-2 appeared to be negative in 8/10 pts (75%). The toxicity was mild with grade 3-4 neutropenia in 2 pts (14%). CMV infection was observed in one pt. Arm R-CHOP: Thirteen pts (93%) achieved CR and 1 resulted non responder. Out of all 13 pts in RC: 1 died in CR for infection, 1 relapsed after 23 months. After a median follow up of 25 months, 12 (86%) pts are alive, 11 (78%) of which are in continue CR. Grade 3-4 neutropenia was observed in 4 (28%) pts. At the end of treatment bcl-2 appears to be negative in 6/9 pts (66%). *Conclusion*. Our data demonstrate that both frontline R-FND and R-CHOP treatments produce high rate of response in terms of CR, OS and molecular remission and low toxicity. A more prolonged follow-up will be needed to determine the long-term efficacy of these combinations.

1191

T(14;18), P53 AND RAS GENE MUTATIONS IN PATIENTS WITH RESIDUAL LYMPHOMA CELLS

B. Cikota, L.J. Tukic, O. Tarabar, Z. Magic

Military Medical Academy, BELGRADE, Serbia and Montenegro

PCR analysis of rearranged antigen receptor genes reaches sensitivity of 10^{-6} and has been demonstrated as valuable tool for detection of minimal residual disease (MRD) in lymphoid malignancies. However, the finding that patients with evidence of MRD sometimes remain in long-lasting remission directs further investigations toward biology of residual disease. The aim was to correlate MRD results with the incidence of relapse and DFI, respectively. Furthermore, the presence of P53 and RAS gene mutations and t(14;18) was analysed in patients with residual malignant cells. The study included 40 B-NHL patients diagnosed and managed in MMA. 13/40 patients had high- (HG) and 27/40 had low-grade (LG) lymphoma. Seven patients achieved partial (PR) and 33 patients achieved complete clinical remission (CCR) after chemotherapy. Peripheral blood samples were analysed for MRD at up to ten follow-up points. All analysis included PCR amplification followed by appropriate electrophoresis. MRD was found in 13/33 patients (12 LG and 1 HG) who achieved CCR. The incidence of relapse was significantly higher in MRD$^+$ vs MRD- B-NHL patients (Fisher's exact test, p=0.0083). In the LG group significant difference was not found. The only MRD$^+$ HG patient relapsed. Significant difference in DFI between MRD$^+$ and MRD- patients was not observed. Concerning MRD+ patients in CCR and patients who achieved PR, t(14;18) was found in six patients (4 relapsed). In the same group P53, K- and N-RAS mutations were not found. H-RAS mutations were found in six patients - 3 relapsed and 3 remains in CCR. Our results demonstrated positive correlation between MRD - positivity and incidence of relapse in B-NHL patients, but didn't indicate significance of P53 and RAS mutations for evaluation of residual clone malignancy.

1192

DOSE-ADJUSTED EPOCH-RITUXIMAB IS HIGHLY EFFECTIVE AND TOLERABLE IN UNTREATED POOR-PROGNOSIS DIFFUSE LARGE B-CELL LYMPHOMA: RESULTS OF A PROSPECTIVE STUDY

J. García-Suarez, M.H. Bañas, M. Lopez Rubio, D. De Miguel, T. Pascual, M.A. Calero, Y. Martin Guerrero, C. Burgaleta

Hospital Prncipe de Asturias, ALCALA HENARES, Spain

Less than 50% of patients with poor-prognosis diffuse large B-cell lymphoma (DLBCL), defined as score 2 and 3 (intermediate-high or high) according to the age-adjusted International Prognostic Index (aaIPI), remain disease-free for lengthy periods. The optimal therapeutic strategy for these patients is still much debated. This study was to evaluate the efficacy and toxicity of Rituximab and dose-adjusted EPOCH (DA-EPOCH-R) regimen for untreated poor-prognosis DLBCL. DA-EPOCH-R regimen (doxorubicin, vincristine, etoposide over 96 hours' infusion with bolus cyclophosphamide, prednisone, and rituximab) was administered to 31 consecutive patients with previously untreated poor-prognosis DLBCL. At the end of DA-EPOCH-R, consolidation radiotherapy (36 Gy) was given to areas of previous bulky disease (≥10 cm). The last 8 patients received an intensive central nervous system (CNS) prophylaxis consisting of 3 courses (after cycles 2, 4 and 6 of DA-EPOCH-R) of orally dexamethasone (40 mg on days 1-4) and intravenous high-dose methotrexate 3 g/m^2 (1.5 g/m^2 in patients > 60 years of age), 12-h infusion, day 1 (with folinic acid rescue). Median cycles of EPOCH regimen administered were 6 (ranged from 2 to 8 cycles). Younger patients (aged < 60 years) required higher dose rates than older patients (>60 years) to achieve the targeted absolute neutrophil count (ANC) nadir. Two-hundred and six cycles of chemotherapy were administered to 31 patients. Of the 31 patients enrolled in the study, 28 were evaluable for response. Overall, 92% of patients had an objective response; 78% (22/28) achieved a complete response (CR) and 14% (4/28) had a partial response (PR) at the end of therapy. At a median follow-up of 23 months (range 9-45), the event-free survival (EFS) and overall survival (OS) were 71% and 85%, respectively. Two CR patients (both with an aaIPI score of 3) relapsed. Only aaIPI score of 3 demonstrated to have an adverse prognostic value. Toxicity at least grade 3 according to the WHO toxicity criteria (incidence by cycle) were: neutropenia (53%), thrombocytopenia (25%), anemia (13%), and oral mucositis (6%). Severe infections occurred in 25% of the cycles in patients older than 60 years of age compared with 10% of cycles in patients younger than 60 years of age. Four patients with previous cardiac disease and 3 patients with HCV antibody showed no severe cardiac nor hepatic toxicity during chemotherapy. There were 2 toxicity-related deaths (treatment-related mortality, 6%): one patient had an early toxic death due to neutropenic sepsis at week 16, and the other patient had a late toxic death due to secondary acute myeloid leukemia (FAB M4) that occurred at 8 months after the end of DA-EPOCH-R. The data from our institution are promising and add to the available evidence supporting the efficacy and safety of DA-EPOCH-R therapy for the treatment of poor-prognosis DLBCL, especially in patients with an aaIPI score of 2.

1193

CHOP VS. RITUXIMAB-CHOP IN DIFFUSE LARGE B-CELL LYMPHOMA: A RETROSPECTIVE COMPARISON OF RESPONSE RATES AND OUTCOME

D. Mihou,[1] P. Konstantinidou,[2] D. Markala,[2] F.R. Patakiouta,[2] C.H. Chatziaggelidou,[2] D. Krikelis,[2] N. Constantinou[2]

[1]*'Theagenion' Cancer Center, THESSALONIKI, Greece* [2]*'Theagenion' Cancer Center, THESSALONIKI, Greece*

Backgrounds. Rituximab is an anti-CD20 monoclonal antibody that

induces cytotoxicity via antibody-dependent cell-mediated and complement-dependent mechanisms, as well as via direct apoptotic signaling. Combination of rituximab with chemotherapy has an additive or synergistic effect and has been reported to increase response rates and prolong remission and survival in patients with diffuse large B-cell lymphoma (DLBCL). *Aim*. To evaluate and compare retrospectively the response rates and outcome of a large number of patients with DLBCL according to the kind of treatment administered, CHOP alone or rituximab-CHOP. *Methods.* Between 1997 and 2004, 204 consecutive patients were diagnosed with DLBCL in our department. Patients were divided in two groups according to the kind of treatment administered. Group A comprised 113 (55.4%) patients, that received CHOP and CHOP-like regimens every 3 weeks and group B consisted of 91 (44.6%) patients, that additionally received rituximab 375 mg/m^2 IV on day 1 of each chemotherapy cycle. Patients in both groups underwent a median number of 6 (1-8) cycles. Radiotherapy was additionally administered in 24 (21.2%) patients of group A and in 28 (30.8%) patients of group B ($p > 0.05$). Patients' characteristics (gender, age, nodal or extranodal primary site of origin, stage, IPI, presence of B symptoms, extranodal involvement other than primary, bulky disease and bone marrow infiltration), as well as response rates, were compared between the two groups using χ^2 tests. Disease-free survival (DFS), overall survival (OS) and failure-free survival (FFS) were estimated according to the Kaplan-Meier method. Differences in survival rates were assessed using the log-rank test. *Results.* Patients were well-balanced regarding their characteristics ($p>0.05$). Median follow-up time for groups A and B was 62 (1-99) and 29 (1-62) months respectively ($p<0.001$). On an intention- to-treat basis, complete response rates were similar between groups A and B (88.5% vs. 89% respectively, $p>0.05$). Actuarial 3-year DFS rate was significantly higher in group B compared to group A (89.4% vs. 72.6% respectively, $p=0.046$). Actuarial 3-year OS and FFS rates were not significantly different between groups A and B (77.7% vs. 70% and 62.5% vs. 69.7% respectively, $p>0.05$). *Conclusion.* According to our results, the addition of rituximab to chemotherapy yields a higher DFS rate than chemotherapy alone, in patients with DLBCL. Nevertheless, our study failed to confirm the superiority of the rituximab-chemotherapy combination in terms of OS and FFS rates, probably due to the significantly shorter follow-up of this group of patients.

1194

MYELOABLATIVE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN POOR PROGNOSIS PATIENTS WITH ADVANCED DIFFUSE LARGE B-CELL AND FOLLICULAR LYMPHOMA EFFECTIVE THERAPY IN FIRST REMISSION

T. Papajik,[1] L. Raida,[1] E. Faber,[1] V. Prochazka,[1] J. Vondrakova[1], S. Rozmanova,[1] Z. Kubova,[1] M. Jarosov,[1] L. Kucerova,[1] J. Jarkovsky,[2] L. Dusek,[2] K. Indrak[1]

[1]*University Hospital Olomouc, OLOMOUC, Czech Republic;* [2]*Masaryk University, BRNO, Czech Republic*

Backgrounds. Conventional chemotherapy in advanced, poor-prognosis diffuse large B-cell (DLBCL) and follicular lymphoma (FL) is still unsatisfactory, and significant number of patients ultimately dies from their disease. The addition of rituximab to initial combination chemotherapy (e.g. R-CHOP and R-CVP) increases the number of complete remissions (CR), prolongs duration of response and survival, but the overall results in high-risk prognostic groups are suboptimal. *Aims.* Number of studies confirm that high-dose chemotherapy and autologous stem cell transplantation (AT) in younger pts. with accumulation of several adverse prognostic factors improves their outcome and prolong survival, and latterly that the treatment with rituximab may be safely included in a chemotherapy regimen preceding stem cell harvest, high-dose chemotherapy and AT. *Methods.* Between 1997 and 2005, a total of 75 newly diagnosed pts. (44 women, 31 men) with poor-prognosis FL and DLBCL were intensively treated (anthracycline-based therapy) at our department. Chemotherapy with addition of rituximab was administrated in 33 of them (44%). 24 pts. achieved complete remission (CR) and 51 pts. partial remission (PR), mostly with a tumor reduction greater than 75%. After BEAM conditioning therapy, median of $7{,}2\times10^6$kg (range, 2,1 - $37{,}3\times10^6$/kg) CD34$^+$ peripheral blood stem cells were reinfused. *Results.* At 100 days following AT, 49 pts. were in CR, 14 pts. in CRu, 7 pts. in PR and 1 pt. relapsed. 4 pts. were shortly after AT and could not be assessed. 15 pts. (20%, 5 with FL, 10 with DLBCL) relapsed/progressed after a median time of 25 months from AT and 8 pts. died from recurrent lymphoma. Only one of the relapsed pts. was treated with rituximab initially (1/33 = 3%), other 14 relapsed pts. were treated with chemotherapy (14/42 = 33%). 60 pts. are still alive in a remission with median follow-up of 34 months (range, 9-117 months) from diagnosis. Estimated 2 years overall survival and event free survival rates are 94% and 87%, respectively, and there were revealed no statistical differences between DLBCL and FL pts. *Conclusions.* Myeloablative chemotherapy and autologous stem cell transplantation in poor prognosis pts. with advanced DLBCL and FL can lead to long-lasting CR. The standard administration of a front-line immunochemotherapy with rituximab can further improve the quality of remission and prolong event-free and overall survival.

Funding: Supported by VVZ MSM 619 895 9205 grant.

1195

CNS LYMPHOMA AND THE USE OF INTRATHECAL RITUXIMAB- REPORT OF THREE CASES

I. Savic,[1] I. Urosevic,[1] V. Uzurov,[1] S. Popovic,[1] M. Zikic,[2] V. Cemerikic-Martinovic[3]

[1]*Institute for Internal Disease, NOVI SAD, Yugoslavia;* [2]*Clinic of Neurology, NOVI SAD, Yugoslavia;* [3]*Histolab Laboratory, BELGRADE, Yugoslavia*

Backgrounds. Central nervous system(CNS) involvment is an adverse prognostic factor for patients with non-Hodgkin's lymphoma(NHL). Because of the limited passage of rituximab through the blood-brain barrier, intrathecal administration of rituximab has been considered as a possible treatment for CNS lymphoma. Methods. 3 patients with recurrent or persistant CD20+ primary parenchymal CNS NHL were treated at Clinic of Hematology Novi Sad.Nine planned intrathecal injections of rituximab at 20 mg dose were given over a 5-week period.One injection was given in the first, then twice weekly for 4 weeks.Injections were administered in 2 mL of 0,9% saline during 2 minutes.Safetly and tolerability were evaluated by clinical evaluation, including neurologic examination, laboratory blod and cerebro-spinal fluid (CSF) tests and adverse-event reporting.Toxicity was graded according to version 2. of the common toxicity criteria(CTC) of the cancer therapy evaluation programm.Tumor response was assessed by weekly CSF cytology, neurologic examination twice weekly,magnetic resonance image(MRI) scanning and immunohistochemical analyses of CSF(at 5 weeks compared with baseline). MRI and physical and neurologic examinations were repated at 6 weeks(4 week after final injection). *Results.* Our preliminary results suggest that intrathecal administration of rituximab was well tolerated (longest interval follow-up is 4 months).Toxicities observed include mild parasthesias occuring in 1 patient.All patients exhibited cytological and biochemical response, without CD20$^+$ lymphoma cells detectable in CSF with clinical remission and no MRI evidence of brain parenchiaml disease. *Conclusions.* Intrathecal rituximab administration represents a novel means of tretment of CNS involment of NHL.Efficasy and safety data are promising, but future trials and follow-up are required to evaluate this route of administration.

1196

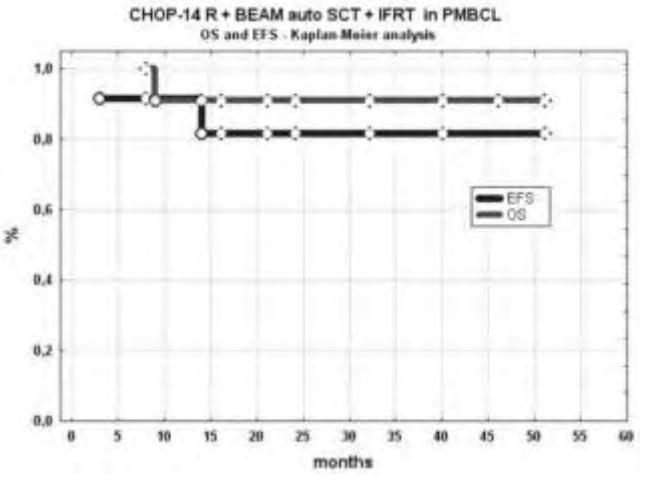
GOOD RESPONSES OF PRIMARY MEDIASTINAL B CELL LYMPHOMA (PMBCL) AFTER CHEMOIMMUNOTHERAPY (CHOP-14-RITUXIMAB) CONSOLIDATED BY BEAM AUTO SCT AND IFRT

W. Jurczak, B. Piatkowska-Jakubas, A. Giza, D. Krochmalczyk, J. Wegrzyn, P. Mensah, D. Hawrylecka, A.B. Skotnicki

Department of Haematology, CRACOW, Poland

PMBL is a distinct entity in WHO classification prior described as DLBCL variant. It presents as mediastinal bulky tumor, locally invasive to adjacent mediastinal structures. The bone marrow is involved only in 2% of cases. Relapses tend to be extranodal, including central nervous system, liver, kidneys. Prognosis in PMBL treated by CHOP regimen is poor in most cases resistance of lymphoma cells occurs already during the first line chemotherapy and 5 year overall survival is about 20%. Patients characteristics. In 2003-2005 12 PMBCL patients were treated at Hematology Department in Krakow. The medium age patient group was 37,2. In 9 cases the disease was limited to mediastinum (stage II according Ann Arbor), and subsequent 3 patients had more advanced disease with a spread to vertebral column, lungs or adjacent muscles. B symptoms were present in all cases. None of the patient had bone marrow involvement. The majority of patients had elevated LDH (medium 901/uL) and bulky disease at diagnosis (mediastinal mass (>20cm) was present in 7 patients, more than 30 cm in 3 patients). IPI was a poor outcome predictor, as it was low (0-1) in 10 cases and intermediate (2-3) in 2 cases. *Treatment schedule and results*. Patients with PMBL were treated either with intensive chemoimmunotherapy CHOP-14-Rituximab according GLSG (10 patients) or ACVBP chemotherapy according GELA (2 patients). In 8 patients - a good partial response to first line chemotherapy was consolidated by BEAM conditioned by auto SCT. All patients

received IFRT. Although further tumor regression after the transplant was moderate, so far 5-50 months after transplantation PFS is 87%. Four further patient were not transplanted due to denial, ineffective stem cells collection or poor performance status. - 2 of them are in CR , and a 3-rd one in a non progressive PR (residual mass ?). Residual masses observed in most of the patients (8/12) at the end of the first line therapy. In the whole group 2 year OS and EFS are 80 and 95% respectively. *Conclusion*. Intensive chemoimmunotherapy does change the prognosis of PMBCL patients although the role of transplant as the first line therapy remains debatable. Effect of radiotherapy is not proven, however similarities between PMBCL and Hodgkin Disease (gene expression analysis, common residual masses at the end of therapy, usually localized disease) make it a tempting therapeutic option.



1197

NATURAL KILLER/T-CELL LYMPHOMA: A SERIES OF SIX CASES FROM A SINGLE WESTERN INSTITUTION

E. García-Garre, S. Rosello, S. Furio, M. Tormo, A. Ferrandez, J.C. Hernandez-Boluda, C. Solano, M.J. Terol

Hospital Clinico Universitario, VALENCIA, Spain

Backgrounds. NK/T-cell lmphoma is a rare entity mostly being reported in Asian countries; representing 6% of total of lymphomas. In western countries its incidence is lower and not well described. Neoplastic cells show an angiocentric pattern of infiltration and usually express CD2, CD3e, CD56, TIA and granzyme B. *Aim.* we described a series of six Caucasian patients diagnosed of NK/T 'cell lymphoma at our institution in Spain in the last 10 years, representing 0.8% of all patients. *Patients and Methods.* median age was 58 years(from 36 to 73), male sex 5/6 (83%). Five patients presented with involvement of nasal and paranasal cavities and one has a non-nasal type. Patients with nasal involvement presented with nasal obstruction and bleeding. B symptoms were present in 2/6 (33%) patients, High LDH 3/6 (50%). Five of the five patients tested had a positive Epstein-Barr serology. Clinical Ann Arbor staging was: I 3 (50%), II 1 (17%) III 1 (17%) IV 1 (17%) and a modified staging system for paranasal lymphomas was T1 2 (33%) T3 1 (17%)T4 2 (33%). International prognostic index was: low risk 4 (66%), low-intermediate 2 (33%). A modified international index was score 0: one patient, 1 two, 2 two and 3 one patient. 3 out of six patients received chemotherapy including anthracyclines (mostly CHOP) as front line therapy and 2 received radiotherapy and one chemoradiotherapy as a rescue for progression following front-line chemotherapy. Results. three of the five evaluable patients achieved a complete remission to front line chemotherapy, one achieved a partial response and one of them progressed immediately after chemotherapy (1) and radiotherapy (1). One patient progressed four months after finishing treatment. 2 of the six complete responders relapsed at three and six years after achieving response, all of them with disseminated disease and died as a consequence of lymphoma. Only 2 of the six patients remain alive at the moment. *Conclusion*. NK/T lymphoma patients diagnosed at our institution presented with clinical features and an aggressive outcome comparable to those described in eastern countries.

1198

HIGH-DOSE CHEMOTHERAPY WITH TANDEM AUTOLOGOUS TRANSPLANTATION IN RELAPSED/REFRACTORY HODGKIN'S DISEASE - A SINGLE CENTER EXPERIENCE

A. Lojko, L. Gil, A. Czyz, M. Kozlowska-Skrzypczak, K. Sawinski, M. Komarnicki

Clinic of Haematology, POZNAN, Poland

Backgrounds. High-dose chemotherapy with autologous stem cell transplantation (auto-HSCT) is commonly used in relapsed/refractory cases of Hodgkin's disease (HD). *Aim.* We report the results of tandem auto-HSCT in patients with relapsed/refractory HD. *Methods.* Thirteen patients were included in this study. The median age was 26 years (range 20-39). Disease status at first auto-HSCT was refractory relapse (n=4) or primary refractory (n=9). Before tandem transplantation all patients received ≥ 2 lines of chemotherapy, one patient received radiotherapy, two relapsed after previous auto HSCT. In eleven patients, only peripheral blood cells were used and in two patients both bone marrow and peripheral stem cells were used. Conditioning regimen with dexamethason, BCNU, etoposide, cytarabine, melphalan (DexaBEAM) was used for the first transplant and busulfan and cyclophosphamid (BuCy2) in ten patients, and treosufan and cyclophosphamid in three patients for the second transplant. *Results.* Hematological reconstitution was complete in all patients at both transplants. The median time to neutrophil recovery (absolute neutrophil count ≥0,5G/l) after first and second transplant was respectively: 11 days (range 7-16) and 12 days (range 9-16) and platelet recovery (platelet count ≥20G/l) was respectively: 14 days (range 10-19) and 21 days (range 12-60). After first transplant only neutropenic fever and confirmed bacterial/fungal infections were observed, treated with good results. After second transplant one patient developed transient congestive heart failure with ventricular arrhythmia and veno occlusive disease was recognized in two patients. One patient (8%) died due to treatment-related toxicity (veno-occlusive disease). In the treosulfan group no serious complications was observed. With the median follow-up of 42 months (range 12-71) ten patients are alive (77%), eight are in remission (61%), four patients relapsed (23%). *Conclusion*. Dose-intensive chemotherapy with tandem transplantation is an option in selected patients with resistant/refractory HD who have poor prognosis and limited treatment opportunity.

1199

IFOSFAMIDE PLUS VINORELBINE SALVAGE THERAPY FOR REFRACTORY OR RELAPSED HODGKIN LYMPHOMA: 23 CONSECUTIVE CASES FROM A SINGLE TEAM

B. Kapás,[1] S. Lueff,[1] P. Reményi,[1] A. Bátai,[1] I. Bodó,[1] Z. Csukly,[1] V. Goda,[1] J. Jánosi,[1] G. Kriván,[2] N. Lovas,[1] S. Nahajevszky,[1] M. Peto,[1] M. Réti[1], A. Sipos,[1] T. Masszi[1]

[1]*National Medical Centre, BUDAPEST, Hungary;* [2]*St. Lszl Hospital, BUDAPEST, Hungary*

Backgrounds. High dose ifosfamide and vinorelbine is an active regimen in the treatment of refractory or relapsed Hodgkin lymphoma (Bonfante et al, 1998). *Aims.* To evaluate the efficacy and toxicity of ifosfamide and vinorelbine therapy in a heavily pretreated patient population before or after autologous stem cell transplantation (ASCT). *Patients*. Twenty-three patients were treated between 2000 Nov and 2006 Feb. The median age at the time of treatment was 28 (18-44) years. The combination was used as first salvage therapy in 11 patients following ASCT for resistant disease or relapse, whereas 12 patients received this treatment prior to or without ASCT. All of these 12 patients were treated with ifosfamide and vinorelbine as third or fourth-line therapy. Six of them had primary progressive disease, 3 had early relapse and 3 had late relapse after standard dose therapy. *Methods.* Ifosfamide (3 g/m^2 days 1-4 by continuous infusion) and vinorelbine (25 mg/m^2 i.v. days 1 and 4) were administered with G-CSF support and uromitexane uroprotection. The median number of the therapeutic cycles was 2 (range 1-9). *Results.* The response rate was 65%, with 11 complete (CR 48%) and 4 partial remissions (PR 17%). Of the 15 responding patients, 8 received ASCT, 4 underwent allogeneic stem cell transplantation with reduced intensity conditioning (NSCT), 2 received further chemotherapy because of progression and 1 had no more therapy and remained in long-term (56 months) complete remission. The regimen was successfully used to mobilize peripheral stem cells in 8 patients (the median number of collected CD34$^+$ cells was 5,05×10^6/kg), while 3 patients did not mobilize. The main toxic effect was grade IV neutropenia, documented in 96% of cycles, non-haematologic toxicity was mild. *Conclusions.* The combination of ifosfamide and vinorelbine proved to be effective to minimize tumor burden before ASCT and NSCT with tolerable toxicity profile. One of our patients

who relapsed following ASCT and had no donor, has remained in continuous complete remission for 56 months.

1200

COMPARISON OF THE EXPRESSION OF DIFFERENTIATION MARKERS BETWEEN DIFFUSE LARGE B-CELL LYMPHOMA OF NODAL AND EXTRANODAL PRIMARY ORIGIN

V. Cemerikic-Martinovic,[1] T. Terzic,[2] M. Perunicic-Jovanovic,[3] L.J. Jakovic,[3] Z. Bogdanovic,[1] S. Knezevic-Usaj[1]

[1]*Histolab, BELGRADE, Serbia and Montenegro;* [2]*Medical School, BELGRADE, Serbia and Montenegro;* [3]*Clinical Centre, BELGRADE, Serbia and Montenegro*

Backgrounds. Diffuse large B-cell lymphoma (DLBCL) is a biologically and clinically heterogeneous lymphoma. DLBCL can be divided into prognostically important subgroups with germinal B-cell (GCB) and activated B-cell (non-GCB) types with a favorable and an unfavorable prognosis using a cDNA microarray. The expression pattern of differentiation markers CD10, BCL6 and MUM1 by immunohistochemistry (IHC) has been proposed as a surrogate to distinguish GCB from non-GCB type. *Aims.* The purpose of our study was to compare the expression frequencies of a panel of B-cell differentiation markers and proliferation rate in DLBCL according to the primary site, lymph node, or different extranodal organs. *Methods.* This study included 50 cases *de novo* DLBCL; 25 of nodal origin and 25 of primary extranodal origin. Sites of extranodal disease were: stomach (8), spleen (6), testis (4), skin (2), ovary (1), pancreas (1), lung (1) and large bowel (1). All the tissue samples were formalin-fixed paraffin sections obtained by biopsy before chemotherapy. The tumors were subclassified according to WHO classification and evaluated by IHC. To define each case as GCB and non-GCB type a panel of 3 antigens, CD10, bcl-6 and MUM-1 was evaluated following the algorithm reported by Hans CP et al (Blood.2004;103;275). All samples were further analyzed for the expression of bcl-2. Immunoreactivity was judged to be positive if 20% or more tumor cells were stained. The proliferation rate was evaluated by percentage of Ki-67 positive tumor cells. *Results.* All tumors were CD20 positive. In nodal DLBCL CD10, bcl-6 and MUM-1 were positive in 17/25 (68%), 15/25 (60%) and 16/25 (64%) cases. Nine cases (36%) were classified into GCB type and 16 (64%) into non-GCB type. Ten cases (40%) were bcl-2 positive, all of non-GCB type. Between extranodal DLBCL CD10, bcl-6 and MUM-1 were positive in 19/25 (76%), 7/25 (28%) and 9/25 (36%) cases. Sixteen cases (64%) were classified into GCB type and 9 (36%) into non-GCB type. Eight cases (32%) were bcl-2 positive, all of non-GCB type. Non-GCB lymphomas were located in skin (both were *leg type* DLBCL), testis, ovary, liver and large bowel. The proliferation rate was higher in nodal DLBCL, with a median of 63.2% of tumor cells Ki-67 positive (range from 30% to 80%). In extranodal group the average proliferation rate was 37.6%. The highest proliferation rate (70-80%) was observed in skin and liver DLBCL. *Conclusion*: In the present series, non-GCB phenotype occurred frequently in nodal DLBCL and were associated with overexpression of bcl-2 and high proliferation rate. In primary extranodal DLBCL non-GCB phenotype, overexpression of bcl-2 and high proliferation was observed in skin *leg type* DLBCL, testicular, ovary and liver DLBCL which are known for their aggressive course. The higher frequency of GCB type in other extranodal sites implies that they have a more differentiated cellular origin than nodal DLBCL and favorable prognosis.

1201

T-CELLS DO NOT SUPPORT OSTEOCLASTOGENESIS IN AN *IN VITRO* MODEL DERIVED FROM NON-HODGKIN LYMPHOMA WITH OSTEOLYTIC LESIONS

R. Rizzi,[1] S.C. Colucci,[2] G. Brunetti,[2] A. Oranger,[2] G. Specchia[1], V. Liso,[1] M. Grano[2]

[1]*Department of Hematology, BARI, Italy;* [2]*Department of Anatomy, BARI, Italy*

Bone involvement from hematological malignancies other than multiple myeloma (MM) and adult T cell leukemia (ATL) is an uncommon event. It is characterized by osteolytic lesions, whose underlying molecular mechanisms remain ill defined. With regard to non-Hodgkin lymphoma (NHL), osteolytic lesions have been reported to occur in about 5-15% of all cases, rarely as a presenting manifestation of disease. By contrast, MM associated lytic bone disease, observed in 70-80% of MM patients, is largely investigated. It appears to be regulated by a complex signalling system, that involves the receptor activator of nuclear factor (NF)-kB (RANK), the receptor activator of nuclear factor (NF)-kB ligand (RANKL) and osteoprotegerin (OPG). In particular, RANKL is a potent osteoclastogenic factor, which also modulates immune response, lymphocyte maturation, lymph node organogenesis and, in combination with macrophage-colony stimulating factor (M-CSF), induces osteoclast formation *in vitro*. RANKL is expressed on malignant cells, osteoclasts, bone marrow stromal cells, CD4+ CD8+ thymocytes, and activated T cells. OPG competes with RANK for binding to RANKL preventing its osteoclastogenic effect, and can act as a decoy receptor for TNF-related apoptosis-inducing ligand (TRAIL) exerting an anti-apoptotic effect. A linkage between immunoregulation by T cells and bone loss has been found in MM and other bone loss associated diseases, as we demonstrated by means of an *in vitro* osteoclastogenesis model derived from peripheral blood mononuclear cells (PBMCs) of patients with MM bone disease. In the present study, our aim was to investigate a mode of regulation of bone turnover in lytic involvement from NHL, entailing T cells and secreted factors. We used an *in vitro* osteoclastogenesis model consisting of unfractionated (and parallel T cell-depleted) PBMC cultures derived from two patients, and established according to the methods we described in *Blood, 2004; 104: 3722*. The former patient was affected by diffuse large B cell (DLBC) NHL with osteolysis, the latter had an extranodal DLBC NHL relapsing at bone without evidence of disease elsewhere. The controls were represented by five subjects with non-neoplastic disease, without any skeletal involvement. The patients and the controls gave their informed consent. The results showed the occurrence of spontaneous osteoclastogenesis in the unstimulated unfractionated PBMC cultures derived from the patients; osteoclasts did not form in the cultures from the controls. (The addition of M-CSF and RANKL was not necessary to promote the formation of osteoclasts in the parallel T cell-depleted PBMC cultures). The freshly purified T cells isolated from the patients did not express RANKL, OPG or TRAIL at mRNA as well as at protein level, similarly to the fresh T cells from the controls. We conclude that, in contrast with MM bone disease, T cells of our patients with osteolytic lesions from NHL seem to play no role in the regulation of osteoclastogenesis. Therefore, further investigations are needed in order to better define bone resorption molecular mechanisms in NHL.

1202

THE PREVALENCE OF ANTI HCV ANTIBODIES IN B-CELL NON-HODGKIN'S LYMPHOMA IN CENTRAL ROMANIA

C.M. Munteanu,[1] M. Grigorescu,[2] M. Deac,[3] R. Mihaila,[3] D. Colita,[2] O. Petrascu,[3] C. Cipaian,[4] D. Ureche,[5] M. Busuioc,[5] A. Colita,[2] D. Maries,[6] C. Dobrea,[7] D. Vulcu,[3] M. Maries,[4] F. Sarbu,[4] C. Beca[4]

[1]*University L. Blaga Sibiu, SIBIU, Romania;* [2]*University of Medicine and Pharmacy, CLUJ-NAPOCA, Romania;* [3]*University L.Blaga, SIBIU, Romania;* [4]*County Clinical Hospital, SIBIU, Romania;* [5]*Center of Blood Transfusion, SIBIU, Romania;* [6]*Intermedica Medical Center, SIBIU, Romania;* [7]*Fundeni Clinical Institute, BUCHAREST, Romania*

Backgrounds. Several epidemiological data suggest the involvment of hepatitis C virus (HCV) in the pathogenesis of some B-cell non Hodgkin's lymphomas in areas with high prevalence of HCV infection. *Aims.* To asses the presence of anti HCV antibodies in a cohort of 54 consecutive patients with B-cell-non Hodgkin's lymphoma admitted to the Department of Hematology of our center from October 1997 to December 2005 in the Sibiu County (a region in the center of Romania with 446,000 inhabitans). The control group was a cohort of 8,445 blood donors tested in the period of time from 1995 to 2002. *Methods.* In order to test the presence of anti HCV-antibodies in patients with B-cell NHL a third generation ELISA test was used. The two patients positive for HIV infection were excluded for this study. In the control group a second generation ELISA test was used (from 1995 to 1999) and a third generation one afterwards. *Results.* From the 54 patients with B-cell NHL (age between 22-77 years, male/female ratio 1,25) 13 were positive for anti-HCV antibodies (prevalence=24,07%). In the NHL HCV positive patients the male/female ratio was 0,625. In the blood donors control group, 108 were positive for anti-HCV antibodies (prevalence=1,28%). The prevalence of anti HCV antibodies was significantly higher ($p< 0,01$) in the cohort of B-cell NHL than in blood donors control group. From the 13 B-cell NHL tested positive for anti-HCV antibodies one was healthy carrier, 4 had liver cirrhosis and 8 had chronic hepatitis. We note 2 deaths with clinical and biochemical phenomena of liver failure (one case of B-cell NHL with hepatic cirrhosis and one case of B-cell NHL with chronic hepatitis) related to chemotherapy. *Conclusions.* 1. In the county of Sibiu we found a significantly higher prevalence of anti-HCV antibodies in B-cell NHL compared to blood donors control group, indicative of the fact that HCV may be involved in the ethiopatogene-

sis of B-cell NHL in our area. 2. The high number of cases of B-cell NHL (12 of 54) who associate chronic liver disease produced by HCV infection. 3.The two deaths related to chemotherapy indicate hepatotoxicity in the cases of B-cell NHL associated with chronic liver disease produced by HCV. 4.We consider that antiviral therapy is required in all this cases to avoid the hepatotoxicity and possibly to induce by itself a regression of the lymphoma (based on the references in literature).

1203

CYTOMEGALOVIRUS REACTIVATION DURING ALEMTUZUMAB THERAPY FOR CLL: SAFETY AND EFFICACY OF VALGANCICLOVIR

A. Lucania, M.R. Villa, S. Improta, M. Esposito, M. Sagristani, A. Carola, A.A. Quirino, L. Mastrullo

PO San Gennaro, NAPLES, Italy

Background and Aims. Several study described a variable incidence of cytomegalovirus (CMV) reactivation in patients treated with alemtuzumab. No prospective reports currently provide results of oral valganciclovir as pre-emptive therapy in patients with CMV reactivation during alemtuzumab treatment. We explored the efficacy and safety of oral valganciclovir as a therapy of CMV reactivation and of prophylaxis of CMV disease. *Methods.* Starting from May 2004, we treated 10 patients (9 males and 1 female; median age 57). Six patients were in partial response after previous chemotherapy regimen containing fludarabine, and 4 were refractory to previous treatment (range 1-7). All patients received alemtuzumab at 10 mg as target dose, 3 times weekly for a prolonged period of 18 weeks. The drug was delivered subcutaneously and, in order to further minimise adverse local therapy-related effects and make the treatment more manageable, were associated with 50 mg of hydrocortisone s.c. for the first two weeks. At baseline all patients had undetectable CMV DNA but were positive by serology. Prophylaxis with oral acyclovir 800 mg bid was given during therapy and for a months after alemtuzumab therapy. CMV reactivation was detected weekly in peripheral blood mononuclear cells by PCR and was considered positive if >200 copie/mL. CMV disease was diagnosed from the association of clinical symptoms with virologic confirmation of a CMV infection of an organ. *Results.* During the treatment 4 patients (40%) showed CMV reactivation. 2 out 4 patients showed fever but no clinical evidence of CMV disease. CMV reactivation appeared after a median of 5 weeks (range 4-6) of treatment. The alemtuzumab and acyclovir prophylaxis were discontinued and the patients were treated immediately with oral valganciclovir 900 mg bid. Only one patients required hospitalization for fever. After a median of 14 days (range 9-21) of antiviral therapy all patients had achieved negative CMV PCR assays; oral valganciclovir was reduced at 450 mg bid and alemtuzumab treatment were resumed. No myelotoxicity or other side effects were observed during the treatment with oral valganciclovir. None of the 4 patient showed other episode of CMV reactivation after reintroduction of alemtuzumab. *Conclusion*: We successfully use valganciclovir in all patients with CMV reactivation. The response was prompt and there was no progression to CMV disease, no relevant clinical toxicity and unnecessary hospitalization for drug administration. Valganciclovir is effective and safe as CMV prophylaxis in CLL patients treated by alemtuzumab, allowing an easy management of a therapy previously difficult to be routinely used.

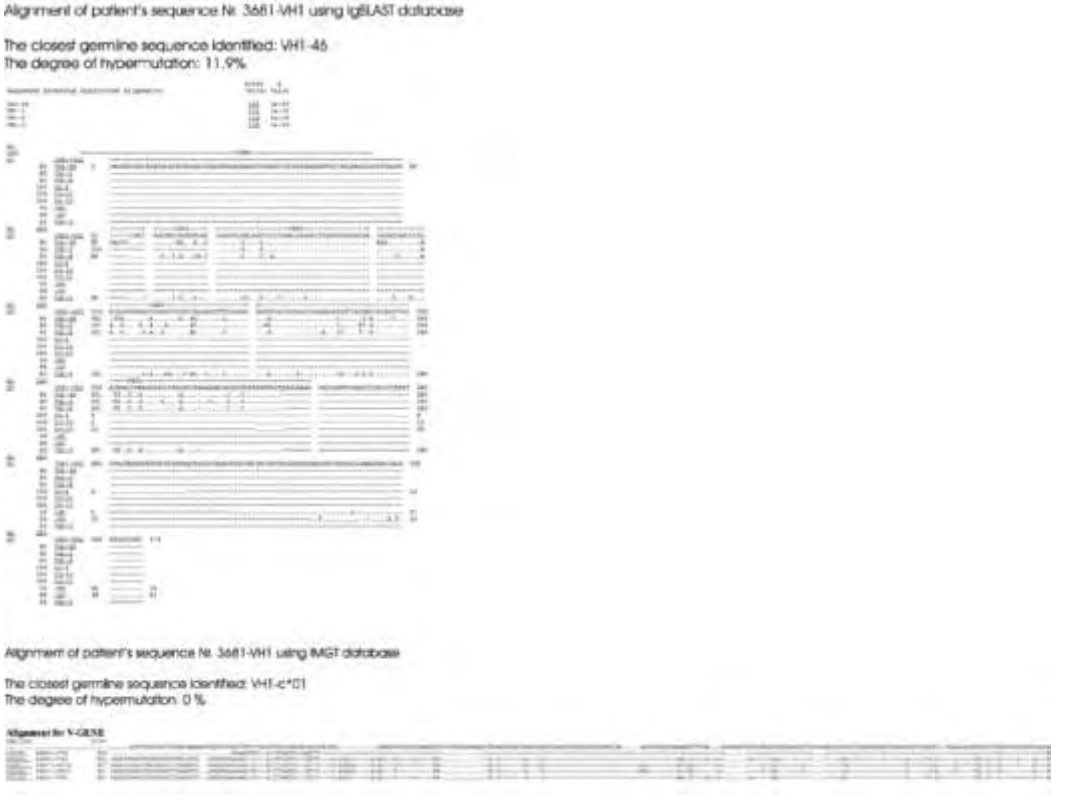
1204

MUTATED OR NON-MUTATED? WHICH DATABASE TO CHOOSE WHEN DETERMINING THE IGVH HYPERMUTATION STATUS IN CHRONIC LYMPHOCYTIC LEUKEMIA?

P.S. Pekova,[1] F. Baran-Marszak,[2] J. Schwarz,[3] V. Matoska[1]

[1]*Hospital Na Homolce, PRAGUE [5], Czech Republic;* [2]*Hpital Avicienne, BOBIGNY CEDEX, France;* [3]*Institute of Hematology & Blood Transf., PRAGUE [2], Czech Republic*

Backgrounds. It has been accepted that the hypermutation status of immunoglobulin heavy chain genes (IgVH) is one of the most important independent prognostic factors in chronic lymphocytic leukemia (CLL). According to the degree of IgVH hypermutation, CLL patients can be stratified into prognostic groups, with favorable or unfavorable prognosis. Aims. Given the impact of IgVH mutation status on clinical setting, it has become highly desirable to standardize the laboratory methodologies used for IgVH mutation status determination. To check the reliability of our laboratory results, we performed an interlaboratory testing, carried out at Homolka Hospital and Hôpital Avicenne. Methods. IgVH hypermutation status was determined in 10 randomly selected CLL patients, according to the Biomed-2 Study protocols. *Results.* From 10 CLL samples tested, in 9 cases identical results were obtained in both laboratories. In one case, the result was discordant. It turned out that the discrepancy was caused not by a technical obstacle, but by the IgVH database used. This finding prompted us to double-check our cohort of 624 CLL patients, using IgBLAST and IMGT databases. The results showed 7.5% (47/624) discrepancies between both databases. In 21 out of 47 cases, the degree of hypermutation has changed in regard to the database used, resulting in major changes in the prognostic subgroup (Figure 1 below). Other irregularities between both databases were identified, with yet to be determined significance. *Conclusions.* In the light of



presented data we would like to stress the necessity to identify/compile the most comprehensive IgVH database to be used for the determination of IgVH mutation status in CLL.

1205

ALLOTRANSPLANTATION FOR CHRONIC LYMPHOCYTIC LEUKEMIA A SINGLE CENTRE EXPERIENCE IMPLYING ITS APPLICABILITY AND CURATIVE POTENTIAL

B. Stella-Holowiecka, T. Czerw, A. Holowiecka-Goral, S. Giebel, J. Wojnar, T. Kruzel, J. Holowiecki

Silesian Medical University, KATOWICE, Poland

It is increasingly clear that allogeneic hematopoetic cell transplantation (alloHCT) offers currently the only curative option for chronic lymphocytic leukemia-CLL, but the relatively high transplant related mortality has limited its application. The recent experience following both the use of newer first line treatment with purine analogues and less toxic pre-transplant preparative regimens appeal for wider trials evaluating alloHCT early in the CLL course in younger patients. *Materials*. Ten patients (F/M=5/5), median age 44,5y (36-53), time from diagnosis to alloHCT 3 years (1-7,5). After diagnosis patients were treated using 1-5 different chemotherapy regimens, all obtained purine analogues and all displayed treatment resistant and progressive course. Other treatments included radiotherapy (n=2), anti-CD20 MoAb (n=2), anti-CD52 MoAb (n=1) and repeated 2 autologous HCT (n=1). The disease status at alloHCT was as follows: CR; n=4, PR;n=3, NR;n=3. AlloHCT characteristics: HLA matched Sibling Donor HCT (n=8), HLA single allele mismatched SibDHCT (n=1), matched Unrelated Donor-HCT (n=1). Stem cell source for SibD transplant: bone marrow -2, peripheral blood -6 (two using positive selection of CD 34+ cells and CD3 cell add back), BM+PB -1, for URD-HCT 'bone marrow in 1 pt. Conditioning: myeloablative Ctx+TBI: n=2; Ctx+TBI+alemtuzumab: n=1; reduced intensity: alemtuzumab (20 mgx5)+fludarabine (30 mg/m 2x5)+melphalan (140 mg/m^2): n=7. The number of transplanted cells: nucleated cells 4,25×10^8 (0,043-12) ; CD34(+) cells 4,34×10^6 (1,6-9,6) ; CD3(+) cells 35×10^6 (15-314) / kg recipient body weight. All transplantations were performed in intensive care, sterile HEPA units. GVHD prophylaxis consisted of cyclosporine A and methotrexate. *Results.* All patients engrafted. Hematopoetic recovery was as follows: granulocytes to 0,5 G/l -22 d (11-55) ; PLT to 50 G/l '24d (13-40). One patient died on day 92 after transplantation of pulmonary Aspergillosis and hepatitis after LPD due to EBV infection transmitted from the donor. The remaining 9 patients achieved CR after transplantation. All 3 patients after myeloablative conditioning acquired full donor chimerism. Among RIC conditioned patients at 6 months 2 displayed full donor chimerism, 3 mixed chimerism and one presented autologous recovery. Acute GVHD grade I was observed in 3/10 patients, limited cGVHD in 3 patients and extensive cGVHD in 2. Six patients developed CMV reactivation, one VZV, and one HBV. Two patients (both after ablative conditioning) died due to late complications: on day 180 (cGVHD with obstructive bronchiolitis) and on day 720 (chronic hepatitis). No patient relapsed with CLL suggesting efficacy of GVL mechanism. At 53 months after transplantation the probability of OS and DFS equals 60% with median observation time of 13 months (7-53). This observation compares well with recent other data (Toze CL et al 2005; 5y OS 39%) and suggests that allotransplantation offers an effective treatment with curative potential for progressive CLL patients who are in good biological condition.

1206

SIGNIFICANCE OF SOME FACTORS IN THE ERA OF MODERN CLL THERAPY

T.E. Bialik,[1] M.A. Volkova,[1] A.A. Molodik,[1] L.Y. Andreeva[1], A.Y. Baryshnikov,[1] T.P. Zogoskina,[2] S.S. Bessmeltsev[3]

[1]*Blokhin Cancer Research Center, MOSCOW, Russian Federation;* [2]*Research Institute of Heamatjlogy and, KIROV, Russian Federation;* [3]*Russian Research Institute Heamatology, ST-PETERBURG, Russian Federation*

Background. Expression of CD38, high level of Bcl-2 and β2-microglobulin and absence of CD95 expression are well-known unfavourable prognostic factors (UPF) for overall and progression free survival (OS and PFS, respectively). It is uncertain whether they retain their significance in the time of fludarabin (F) and mabthera (Rituximab (R) therapy. *Aim*. To evaluate the influence of the above mentioned prognostic factors on clinical course of CLL in patients (pts) treated with modern therapy. *Patients and methods*. Sixty nine pts with B-CLL were included in this study (median age 59,5 years; Binet stage A - 1, B - 41, C - 27; median follow up was 143 mo, median follow up after the start of treatment was 43 mo). Thirty four pts received FC treatment - F 25 mg/m^2 and cyclophosphamide (C) 300 mg/m^2 for 3 days; 35 pts received RFC treatment - R 375 mg/m^2 on day 1, FC regimen on days 2-4. All pts received 6 cycles of therapy. The multivariate analysis with Cox's regression model was used. *Results*. 18,5% of pts had all factors investigated, 27,7% had 3 and 21,5% 2 unfavourable factors in different combinations. One factor was found in 26,2% and none in 6,1%. In pts without UPF median OS was 107 mo; in pts with Bcl-2 expression, 97 mo; with Bcl-2 and CD38 expression, 70 mo. High β2-microglobulin level as well as absence of CD95 expression had no prognostic significance. The multivariate analysis showed that expression of CD38 (Relative Risk-RR=0,57, p= 0,3) and high level of Bcl-2 (RR=0,61, p=0,3) had the most pronounced negative influence on OS. For PFS lack of CD95 expression (RR=0,83, p= 0,099) and especially expression of CD38 (RR=1,26, p=0,059) were the most unfavourable factors. Median PFS was not achieved in pts with any UPF combinations without CD38 expression whereas in pts with all 4 UPF it was only 20 mo. *Conclusion*. Modern therapy with FC and RFC allows overcome the negative influence of high level of β2-microglobulin and Bcl-2 and lack of CD95 expression. CD38 expression retains its unfavourable significance.

1207

INFLUENCE OF CLADRIBINE ON BONE MARROW ANGIOGENESIS IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

A. Szmigielska-Kaplon,[1] D. Jesionek-Kupnicka,[1] J. Gora-Tybor[1], J.Z. Blonski,[1] H. Urbanska-Rys,[1] R. Kordek,[2] M. Kasznicki,[3] T. Robak[3]

[1]*Med Univ of Lodz, LODZ, Poland;* [2]*Dept Pathol, Med Univ of Lodz, LODZ, Poland;* [3]*Dept. Hematol, Med Univ of Lodz, LODZ, Poland*

Backgrounds. Angiogenesis is the process of formation of new blood vessels. The process is increased in many neoplastic diseases, including chronic lymphocytic leukemia (CLL). The purine nucleoside analogues, fludarabine and cladribine, represent a novel group of cytotoxic agents with high activity in low grade lymphoid malignancies. Fludarabine decreases bone marrow vessels density in CLL patients. The influence of cladribine on bone marrow angiogenesis in CLL was not studied so far. *Aims.* The aim of the study was to evaluate the influence of cladribine on angiogenesis in bone marrow of CLL patients. *Methods.* Parafin-embaded trephine biopsies were prepared and stained with antibody to CD34 for endotelial cells in patients with CLL before and after treatment with cladribine. Number of microvessels were counted in hot spot places, the areas, with highest vessels density under the microscope in 200x magnification. *Results.* Trephine biopsies from 14 previously untreated progressive CLL patients were evaluated before and after treatment with cladribine. Female/male ratio was 8/6 and median age of the patients 59 years (range 44-73). Staging according to Rai : Rai 0'2 8 patients, Rai 3-4 6 patients. All of the patients received cladribine alone (4 patients), in combination with cyclophosphamide (7 patients) or in combination with cyclophosphamide and mitoxantron (3 patients). All of the patients responded to the therapy and were in complete remission (4 patients) or partial remission (10 patients) according to NCI sponsored Working Group criteria. Median vessels number in hot spot places before treatment was 105 (range 45-238) and after treatment 65 (range 35-1600, p=0,02). There were no differencences between different regimens containing cladribine. *Conclusions.* Number of vessels in bone marrow of CLL patients was decreased after treatment with cladribine containing regimens.

1208

MATURE B-CELL AND T-CELL NEOPLASMS PRESENTING WITH LYMPHOCYTOSIS: A SYSTEMATIC DIAGNOSTIC APPROACH BASED ON CLINICAL, MORPHOLOGIC, IMMUNOPHENOTYPIC AND PATHOLOGICAL FEATURES IN 373 CONSECUTIVE CASES

I. Del Giudice,[1] M.S. De Propriis,[1] F. Mancini,[1] E. Pescarmona[1], I. Della Starza,[1] B. Anaclerico,[1] S. Pileri,[2] A. Tafuri,[1] F.R. Mauro[1], E. Giammartini,[1] A. Pulsoni,[1] A. Guarini,[1] C. Besses[3]

[1]*University La Sapienza, ROME, Italy;* [2]*Hematology & Oncology L.Seragnoli, BOLOGNA, Italy;* [3]*H. Del Mar, BARCELONA, Spain*

Background. Various types of mature B- and T-cell malignancies may involve the peripheral blood (PB) and bone marrow (BM) at presentation. However, among patients requiring an hematological diagnostic work-up because of persistent lymphocytosis, information on the pattern and proportion of the different diagnoses is scanty. *Aims*. To analyze the results of a systematic approach carried out for the differential diagnosis of cases consecutively referred to our center because of persistent

lymphocytosis, focusing in particular on the differential diagnosis of clonal B-cell lymphocytoses. *Methods*. Between January 2003 and December 2004, we evaluated 373 consecutive patients (M/F: 190/183) with an absolute (i.e. >5000/m^3) (81%) or relative (19%) lymphocytosis. Median age was 68 years (range: 19-91). Clinical features, lymphocyte morphology, immunophenotype, BM and/or lymphnode histopathology were reviewed. PB cell morphology was independently reviewed by three experts. Immunophenotype was performed on fresh whole blood samples using four-color immunostaining with a panel of B- and T-cell markers, a FACSCalibur flow cytometer (BD Biosciences) and the Cell Quest software (BD Biosciences). T-cell clonality was assessed by PCR analysis of the T-cell receptor (TCR) γ chain variable regions and using the TCR Vβ Kit (Beckman-Coulter Immunotech, Marseille, France) for the TCRVβ chain families repertoire. Histopathology evaluation of BM and/or lymphnode was performed in cases whose PB morphology and immunophenotype suggested a likely diagnosis of B/T non-Hodgkin lymphoma (NHL) or could not distinguish between B-NHL/chronic lymphocytic leukemia (CLL). *Results.* A B-lineage lymphocytosis was recorded in 81% cases (n=301; 241 CD5+ and 60 CD5-), a T-lineage in 14% (n=52) and a normal lymphocyte pattern in 5% (n=20). In terms of PB morphologic/immunophenotypic criteria, among clonal B-lymphocytoses, 44.5% (n=134) had features of CLL, 44.5% (n=134) were B-NHL, 10% (n=30) were provisionally defined as B-NHL/CLL for intermediate features, 1% (n=3) were hairy cell leukemia (HCL). Of 107 CD5+ cases not fulfilling the standard diagnosis of CLL, 59 underwent BM and/or lymphnode biopsy; 41% were diagnosed as B-NHL with leukemic spillover (5 marginal zone lymphomas (MZL), 4 mantle cell lymphomas, 2 follicular lymphomas (FL), 2 lymphoplasmacytic lymphomas (LPL), 11 unspecified low grade B-NHL) and 59% with CLL. Even among CD5+CD23+ cases not fulfilling the standard diagnosis of CLL, 25% (11/43) proved to be leukemic B-NHL at histopathology evaluation. Of 60 CD5- cases, 37 underwent a biopsy; the final diagnosis was MZL in 21 cases, FL in 4, LPL in 3, HCL in 2, unspecified low grade B-NHL in 7. In CD5+ B-NHL and CD5- B-NHL, the expression of CD38 (p<0.001) and adhesion molecules (CD11a, CD18) (p<0.001) were significantly higher than in CLL, as well as the presence of superficial adenopathy (p<0.001), splenomegaly (p<0.001), thrombocytopenia (p<0.05) and raised LDH (p<0.001). Among T-NK lymphocytoses, 45 cases showed a T-LGL expansion (reactive in 21, clonal in 22), 2 NK-LGL, 3 T-NHL, 4 unspecified T-cell lymphoproliferative disorders. *Conclusions*. This study highlights the frequency of various B- and T-cell neoplasms presenting as lymphocytosis and the value of lymphocyte morphology, immunophenotype and histopathology in identifying and subclassifying low grade B-NHL with leukemic presentation. These observations have prognostic-therapeutic implications.

1209

THE USE AND CORRELATION WITH FLOW CYTOMETRY OF FLUORESCENT MOLECULAR BEACONS IN REAL TIME PCR OF IGH GENE REARRANGEMENTS FOR EVALUATION OF MINIMAL RESUDUAL DISEASE IN MULTIPLE MYELOMA

I.O. Kara, A. Yigin, B. Sahin, S. Paydas

Cukurova University Faculty of Medicine, ADANA, Turkey

At present, the prognostic value of the amount of residual tumor cells in PB, BM or stem cell harvests and its changes over time is stil not clear. Also the advent of new therapeutic approaches to multiple myeloma made necessary the introduction of novel methods for detection of minimal residual disease. Among others approaches residual disease can be detected by using flow cytometry. The aim of the present study was to evaluate a real time PCR test for the IgH gene using alellespecific molecular beacons as fluorescence probes to quantify residual disease and also correlate flow cytometric detection of plasma cells in MM patients during followup after treatment with high dose of chemotherapy or standart chemotherapy. After clinical diagnosis of 17 MM patients, the CDR1, CDR2 and CDR3 regions of the IgH gene were analysed and sequenced to identify its clonal nature. Unique sequences of the clonal IgH rearrangement were used to design specific molecular beacon probes for each MM patient. We have also examined the co-expression of CD19, CD38, CD45, CD56, and CD138 molecules in cells of bone marrow aspirates in patients with multiple myeloma by flow cytometry. Results. The active disease had been accepted of whom plasma cell infiltration ratio was over 10% in bone marrow and also of whom labeled by CD38 and CD138 by FCM. The detection of the MRD was positive in 13 patients by RT-PCR, respectively. The infiltration ratio was correlated with CD138 expression (p=0.009) and RT-PCR detection of plasma cells (p=0.006) and also significant correlation had been found between RT-PCR detection and CD138 expression respectively. No any correlaton was found between other surface antigens (CD38, CD45, CD56). Our results indicated that real time PCR with specific molecular beacons provides a feasible, accurate and reproducible method for the determination of minimal residual disease in MM. By FCM only CD138 expression may have been used as disease marker in addition of the RT-PCR detection.

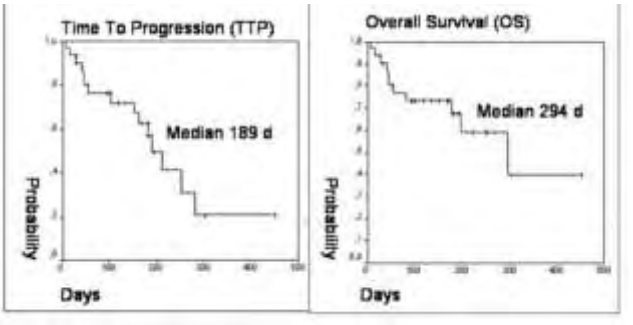
1210

BORTEZOMIB IN COMBINATION WITH HIGH-DOSE DEXAMETHASONE FOR RELAPSED MULTIPLE MYELOMA

L.F. Casado Montero,[1] F. Solano,[2] M.I. Gómez-Roncero,[3] C. Calle,[4] I. Cano,[5] M.A. Foncillas,[6] J.R. Romero[7]

[1]*Hospital Virgen de la Salud, TOLEDO, Spain;* [2]*Hospital Virgen del Prado, TALAVERA, Spain;* [3]*Hospital de la Luz, CUENCA, Spain;* [4]*Hospital Nuestra Senora de Alarcos, CIUDAD REAL, Spain;* [5]*Hospital La Mancha Centro, ALCAZAR DE SAN JUAN, Spain;* [6]*Hospital Santa Barbara, PUERTOLLANO, Spain;* [7]*Hospital General de Albacete, ALBACETE, Spain*

Backgrounds. Single agent bortezomib treatment yields partial responses (PR) in 24% of patients with relapsed, refractory multiple myeloma (MM) and 38% in patients who had received 1 - 3 previous therapies. Dexamethasone (DEX) increases bortezomib anti-myeloma activity. The present study was initiated to study bortezomib in combination with DEX. *Patients and Methods.* 32 patients (pts) with advanced MM were scheduled to receive bortezomib 1.3 mg/m^2 IV days 1, 4, 8, and 11 q 3 weeks for 8 cycles in combination with DEX 40 mg IV or PO on the day of bortezomib injection and the day after. Patient characteristics were median age 66 years; β-2-Mycroglobulin > 3,0 mg/L, 64%; median number of prior therapies 3 (range 2 - 6), and 38% of patients had relapsed after high-dose melphalan. EBMT criteria were used for response definition.



Results. Four pts (13%) achieved a complete response (IF negative), five pts (16%) a complete response (IF positive), 13 pts (38%) a PR, and 0 (0%) a minor response (MR) resulting in an overall response rate (≥MR) of 69%. Median time to disease progression (TtPD) was 189 days (6,3 m). After a median follow-up of 18 months, median overall survival was 294 days (10 m). The median number of cycles administered was 4 (range 1-8). Grade 4 neutropenia was observed in one patient (3%), grade 4 thrombocytopenia in 6 pts (19%), without any thrombocytopenic bleeding. Grade 3/4 non-hematologic toxicities requiring dose or schedule modifications were peripheral neuropathy (6%), neuropathic pain (6%) fatigue (4%), herpes zoster (9%), and cutaneus events (3%). *Conclusions.* Bortezomib in combination with DEX is a highly active regimen without increased toxicity as compared to a single agent treatment with bortezomib.

1211

BONDRONAT IN THE TREATMENT OF BONE LESIONS IN MULTIPLE MYELOMA

S. Stankovic,[1] L. Cevreska,[2] O. Karanfilski,[2] N. Siljanovski,[2] T. Smilevska,[2] Z. Stojanovski,[2] L. Hadzi-Pecova,[2] M. Lozance,[2] A. Ljatifi,[2] A. Stojanovic[2]

[1]*Clinical centre, SKOPJE, Macedonia;* [2]*Department of Hematology, SKOPJE, Macedonia*

Backgrounds. Multiple myeloma (MM) is a malignant disease characterized by skeletal involvement. Osteolytic bone lesions from MM are associated with skeletal complications such as bone pain and pathologic fractures which have a negative impact on quality of life. Bisphosphonates have been shown to decrease the progression of osteolytic lesions,

bone pain and fractures. *Aims.* Aim of this study has been to evaluate the efficiency of ibandronate (Bondronat, F.Hoffman-La Roche) on the course of bone lesions in myeloma patients and also its safety particularly concerning renal function. Methods. We analysed a group of 28 patients in clinical stage III-A or III-B (median age 59.9 years, range 42-77, male: female ratio 13:15) who were currently treated, independently from the adopted chemotherapy, with Bondronat given as an IV infusion over 1-2 hours every four weeks. Patients were clinically evaluated before, during and after Bondronat administration with the median time of observation 7.7 months (range 3-18). Evaluation of osteolytic lesions was performed during the study associated with skeletal complications such as bone pain and pathologic fractures. Routine serum chemical screening including creatinine, calcium, phosphorus and AP were performed. Markers of tubular damage (NAG, AAP, gama-GT and β-2M) were measured in urine before and after Bondronat administration. Results. Clinical improvement of skeletal pains was observed in 23 pts (82%): 12 pts (43%) had a complete pain relief with no more necessity for analgesic-drug use, and 11 pts (39%) had a minor effect, while 5 pts (18%) had no improvement. Nine patients had pathologic fractures at baseline (8 were on vertebral bodies and 1 on ribs) and all of them underwent radiotherapy. During the observed period we didn't find any new pathologic fracture. There were no significant adverse effects associated with the administration of Bondronat. Twenty-one patient (75%) had normal renal function at baseline and the rest had varying degrees of renal insufficiency. No clinically relevant changes in serum creatinine occurred even in patients with existing renal impairment. Transient hypocalcaemia was detected in 3 pts (10%). The levels of NAG, AAP, γ-GT and β-2M were similar before and after administration of Bondronat. Conclusions. The treatment with Bondronat has reduced skeletal morbidity and was well tolerated in our group of myeloma patients; we didn't find any signs of acute renal toxicity in the observed period.

1212

OSTEONECROSIS OF THE JAW IN PATIENTS WITH MULTIPLE MYELOMA DURING AND AFTER TREATMENT WITH ZOLEDRONIC ACID

J.M. Calvo-Villas,[1] M. Tapia Torres,[2] J. Govantes,[2] E. Carreter,[3] F. Sicilia[3]

[1]*Hospital General de Lanzarote, ARRECIFE DE LANZAROTE, Spain;* [2]*Hospital General de La Palma, SANTA CRUZ DE LA PALMA, Spain;* [3]*Hospital General de Lanzarote, ARRECIFE DE LANZAROTE, Spain*

Backgrounds. An increase of the incidence of osteonecrosis of the jaw (ONJ) in subjects with multiple myeloma (MM) receiving zoledronic acid has been reported in recent years. Aims. Based in these reports, we analyze the incidence, the clinical features, and the factors associated with the development of ONJ in patients with MM treated with zoledronic acid. Methods. Sixty-four patients diagnosed with MM and treated with zoledronic acid, alone or after pamidronate, between August 1996 and March 2006 were enrolled in this study. Demographic data, predisposing factors (including dental extractions and oral surgery), the antimyeloma therapy received and the number of courses of biphosphonates and the zoledronic acid were recorded and compared with the results of the published series. The main characteristics of the seven patients with ONJ, including risk factors of the complication, clinical and physical examinations data, diagnostic methods and treatment established were reported. *Results.* The overall incidence of ONJ was 7 out of 64 patients (10,93%). The ONJ has been associated with a recent oral surgical procedure (p<0,0001) and with the prior presence of dental or periodontal pathology (p=0,007). There was no association among the emergence of ONM with age (p=0,536), sex (p=0,547), type of paraprotein (p=0,778), presence of lytic bony lesions (p=0,667), therapy with dexametasone (p=0,128), thalidomide (p=0,564), auto-stem cell transplantation (p=0,317) or receiving >3 different courses of oncologic treatments. The means of infusions of biphosphonates (zoledronate plus pamidronate) and of zoledronic acid before onset of osteonecrosis (SD) were respectively 38,1 (14,7) and 30 (7,0) in contrast to 22,5 (16,4) of biphosphonates and 19,5 (11,8) cycles of zoledronate (p=0,03) in the patients who did not present this complication. The cumulative risk of ONJ increased from 6,7% after 20 treatments with zoledronic acid up to 31,7% at 36 infusions. The site of ONJ was maxilla in three patients and mandible in four. All had received treatment with chemotherapy, dexametasone, thalidomide or auto-stem cell transplantation. Three patients were receiving pamidronate before zoledronic acid. The clinical and examination data were pain in all the cases associated to dental ulcer and local infection in six patients. The diagnosis was confirmed in all the cases with panoramic radiology or CT scan of the jaw, and a biopsy was obtained to exclude metastatic disease in four cases. Zoledronic acid infusions were discontinued in six patients: 3 at the time of diagnosis and 3 after the development of new lesions of ONJ. Three patients exhibited osteonecrotic lesions of the jaw after discontinuing zoledronic acid several months before. All patients received treatment with chlorhexidine rinses, antibiotics and surgical debridement. The follow-up after the diagnosis of ONJ was at least six months. Conclusions. The ONJ in patients with MM who underwent dental or oral surgery appears to be associated with long term exposure to zoledronic acid. A previous dental pathology and the time of exposure to zoledronic acid are main factors in the development of the ONJ. The long-lasting bone effect of biphosphonate could explain the appearance of osteonecrotic lesions after discontinuing treatment with biphosphonate.

1213

RE-TREATMENT WITH BORTEZOMIB IN MULTIPLE MYELOMA

P. Musto,[1] A. Falcone,[2] G. Sanpaolo,[2] P. Scalzulli,[2] M.L. Barone[1], R. Guariglia,[1] C. Musto,[1] L. Ciuffreda,[1] G. Pietrantuono[1], N. Cascavilla[2]

[1]*CROB, RIONERO, Italy;* [2]*IRCCS Casa Sollievo della Sofferenza, S. GIOVANNI ROTONDO (FG), Italy*

Background and Aims. Bortezomib is an effective agent for multiple myeloma, currently licensed for the treatment of relapsed/refractory disease after first line therapy. There are, however, few reports about the use of bortezomib as re-treatment (*re-challenge*) in myeloma patients who have previously received the same drug for their disease. The clinical outcome of five patients with these characteristics is reported. *Methods*. Five patients were re-treated with bortezomib alone or in combination; three were male and two female, with an age ranging from 45 to 66. All patients had a prior salvage therapy with bortezomib or bortezomib plus dexamethasone, after 2 to 4 lines of other chemotherapies, including autologous stem cell transplantation and thalidomide. All patients achieved at least a partial response (reduction of M-component > 50%) after the first treatment with bortezomib and relapsed after 3 to 19 months. *Results*. Bortezomib was re-administered at the standard dose of 1.3 mg/sqm IV, days 1, 4, 8, 11, q3wks in four patients; dexamethasone (20 mg/d for two days after each infusion of bortezomib) was added in 3 out of 4 patients. Severe hematological or extra-hematological toxicities did not occur, but dose reduction or temporary interruption of the treatment were occasionally required, mainly due to moderate thrombocytopenia, neuropathy and skin rashes. After 4-8 cycles, three patients achieved a partial response, with reduction of M-component > 50-75% and concomitant consistant decrease of marrow plasma cell infiltration. Duration of second response to bortezomib ranged from 3 to 8 months. In one patient a stabilization of the disease was obtained. In the fifth patient bortezomib was employed at the dose of 1.3 mg/m^2 days 1 and 4 in combination with melphalan (100 mg/m^2 e.v.), thalidomide (100 mg/d for 5 days) and dexamethasone (40 mg e.v. for 4 days), as conditioning regimen (MVTD) for a further autologous stem cell transplantation. An impressive, rapid complete response with negative serum immunofixation (M-component was 6.1 g/dl before transplant) occurred. This response had a brief length and the patient relapsed after 3 months. The same regimen was given once again and the same complete response was achieved in a few days. The patient, however, died of interstitial pneumonia during the aplastic phase of transplant. *Conclusions*. Our data, although limited, suggest that re-treatment with bortezomib of myeloma patients, who experienced a clinical benefit after the first treatment, is feasible and may induce a new significant response.

1214

A SINGLE CENTER REPORT ON AUTOLOGOUS STEM CELL TRANSPLANTATION FOR PATIENTS WITH MULITPLE MYELOMA

M. Bernardi,[1] J.K. Koenig,[2] O.K. Krieger,[2] M.G. Girschikofsky,[2] D.L. Lutz[2]

[1]*San Raffaele Scientific Institute, MILAN, Italy;* [2]*KH Elisabethinen, LINZ, Austria*

Autologous stem cell transplantation (ASCT) is a recommended treatment option for patients with advanced multiple myeloma (MM). From Oct. 1996 to Aug. 2005 we performed 121 ASCT in 71 MM-patients (age 58 (median:37-67) years; female:31, male: 40). Thirtythree patients (pts) were transplanted with a single course (26 pts. between 1996 -1999 and 7 pts. thereafter due to inelegibility for a second transplant (e.g. late infections (2) , toxic side effects: carditoxicity (2), neurotoxicity (1), dermatitis (1), sMDS (1)). Thirthyeight patients underwent multiple cours-

es of transplantations (26 double and 12 tripple ASCT). No significant differences between both groups were seen according to age, sex or stage of disease at the time of ASCT, but more patients who relapsed after conventional treatment (15 vs. 5 pts) were included in the single than in the multiple ASCT group. Conditioning chemotherapy consisted of Melphalan 200 mg/m^2 for single and double ASCT and 100 mg/m^2 for tripple ASCT. All patients received peripheral stem cells. The hematological recovery (median time to PMN>0,5 G/l : 11(8-13) days and to PLT > 50 G/l : 13 (8-55) days) did not differ between the first or the following transplants. All patients but one in each group responded to transplantation. In the single ASCT group 1 treatment related death occured and in the multiple ASCT group 1 pt. had progressive disease shortly after tandem transplantation. The complete remisson rate (< 5% plasma cells in bone marrow and disapperance of paraproteinamia and/or paraproteinuria) was 42% and did not differ between the two groups (14 /33 pts. vs 16/38 pts.) Although the relapse rate is higher in the single ASCT group (22/33 pts.) than in the multiple ASCT group (18 / 38 pts.) no significant difference could be seen in median progression free survial (25 vs. 28 months) and the median overall survival (69 months vs. not reached yet), caused by a longer observation time for single ASCT (median 42 (1-147) months) than for multiple ASCT (median 25 (5-71) months). Autologous transplantation is a tolerable treatment option even for older patients with multiple myeloma. In 85% of the patients provided for multiple transplantations all courses of ASCT could be performed. Due to heterogeneity of the patients and the different observation periods no final conclusions can be drawn concerning the outcome of the transplantation.

1215

SERUM INTERLEUKIN-17 AND ITS RELATIONSHIP WITH ANGIOGENIC FACTORS IN MULTIPLE MYELOMA

M.G. Alexandrakis,[1] G. Tzirakis,[1] C. Pappa,[1] A. Sfiridaki,[2] R. Alexandraki,[1] M. Kafousi,[3] A. Alegakis,[3] E. Stathopoulos[3]

[1]*University Hospital of Crete, HERAKLION, Greece;* [2]*Venizelion Hospital, HERAKLION, CRETE, Greece;* [3]*Medical School Crete, HERAKLION, CRETE, Greece*

Background. Interleukin-17 (IL-17) is a CD4 T-cell derived mediator of angiogenesis that stimulates vascular endothelial cell migration and regulates production of a variety of proangiogenic factors, such as tumor necrosis factor-α (TNF-α) and vascular endothelial-cell growth factor (VEGF). Angiogenesis is implicated in the progression of multiple myeloma (MM). Overexpression of two potent inducers of angiogenesis - TNF-α and VEGF- has been found in MM cell lines and in the serum of patients with the disease. In MM, bone marrow angiogenesis parallels tumor progression, and is associated with poor prognosis, suggesting an angiogenesis-dependent regulation of disease activity. *Aims*. To date, there are no reports on the role of IL-17 in correlation with angiogenic factors in MM. Aim of the present study was to examine the relationship between serum levels of IL-17 and other angiogenic factors in MM patients. *Methods*. The study population included 40 (19 males, 21 females) newly diagnosed MM patients (median age 66 years, range 37-84). Ten patients had stage I disease, 13 stage II, and 17 had stage III disease according to the Durie-Salmon classification. Fifteen patients had monoclonal immunoglobulin IgGα, 9 had IgGα, 6 patients had IgAα, and 6 had IgAα, whereas the remaining 4 patients had light chain disease. Serum samples from 12 persons, age and sex-matched healthy volunteers, were used as controls. Serum IL-17, VEGF, and TNF-α were measured by solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using monoclonal human antibodies against IL-17, VEGF, and TNF-α from commercially available test kits (Quantikine™, R&D Systems Inc. Minneapolis MN, USA). Blood vessels were highlighted by immunostaining endothelial cells with a monoclonal antibody to CD34. Microvessel density (MVD) was assessed in all patients biopsies and 15 normal bone marrows. *Results*. The mean serum value of IL-17 in the group of MM patients was 27.61 pg/mL; although it was higher than the mean value of the control group (26.34 pg/mL) the difference did not reach statistical significance. VEGF, TNF-α, MVD were significantly higher in the MM group than in the control group (260.5±166.9 vs 82.2±15.9 pg/ml, 22.59±9.88 vs 11.51±3.81 pg/ml, 9.17±5.81 vs 2.32±0.77/0.0625 mm^2, p<0.05 in all cases). Levels of IL-17 VEGF TNF and MVD in MM patients of both stage II and stage III were significantly higher compared to stage I patients (p<0.05). Serum values of IL-17 in MM patients correlated positively with VEGF (Spearman's rho = 0.606), TNF-α (r = 0.552) (p<0.001 in both instances), but also with MVD (r = 0.385, p=0.014). *Conclusions*. These results suggest for a role of IL-17 in the promotion of angiogenesis and associated disease progression in MM and support *in vitro* data that IL-17 induces TNF-α and VEGF production.

1216

EARLY DEATH AND MULTIPLE MYELOMA. EXPERIENCE AT A SINGLE INSTITUTION

V. Jimenez

INCMNSZ, MEXICO CITY, Mexico

Background. Early Mortality after diagnosis of multiple myeloma (MM) is often attributed to combined effects of active disease and comorbid factors. Symptomatic myeloma causes anemia (40%), thrombocytopenia (14%), and neutropenia (6%) at diagnosis. Skeletal disease (70%) reduces mobility, impairs ventilation, and may result in hypercalcemia. Aims. The aim of our study was to assess early mortality associated to infectious complications in patients with newly diagnosed multiple myeloma. Material and methods. We enrolled all patients who fulfilled entire criteria for multiple myeloma between January 1995 and December 2005. The present study is a retrospective, descriptive and observational one. Early mortality was calculated from date of entry onto the trial to date of death or date last seen, as appropriate, and was defined as death within 60 days.Infectious diseases were confirmed clinically or microbiologically. Secondary endpoint was to assess the response rate. It was estimated based on the best response to therapy for each patient during the course of treatment. Statistical analyses were performed using SPSS version 13.0. Fisher's Exact test was used to evaluate the statistical significance of associations in two-way contingency tables. A p value <0.05 was considered as statistically significant. *Results.* Between January 1995 and December 2005, we treated 122 patients with multiple myeloma, 67 patients received VAD (54.9%) , 35 patients received thalidomide and dexamethasone (28.7%) and 20 patients received melphalan/prednisone (MP, 16.4%).The frequency of response (CR, VGPR/NCR, PR) in the group of thalidomide and dexamethasone was 80% (CR,22.8% VGPR/NCR 20% and PR, 37.2%) being higher than VAD, 50.7% (CR 16.4%,VGPR/NCR 5.9%, PR 28.4%). p 0.0005 Early mortality before day 60 occurred in 12 (9.8%) of patients registered onto MM database at our Institution from 1995 to 2005. Patients who died early were shown to be older (> 65 years; p<.0001), have a poorer performance status (p<0.0001), and reduced serum albumin (p<.04) compared with the remainder of the MM database patients. Early mortality was associated with a greater tumor burden and activity (creatinine, β2-microglobulin, albumin, hypercalcemia and C-reactive protein; p<0.0001). There was a significant correlation between early death (ED) and evidence of hematopoietic dysfunction as evidenced by anemia (p .0001), thrombocytopenia (p <0.0001), neutropenia (p=0.04), and lymphocytopenia (p=0.007). Renal function was impaired in twice as many of the early death patients with higher presentation serum creatinine and urea (p< .005). Renal failure was contributory to 4/12 early deaths. Bacterial infection directly caused 11/12 early deaths (91%). Specifically pneumonia occurred in 42 (30%) of 136 bacterial infections (122 patients), and 11/12 patients in the ED group. Generalized sepsis occurred in 18 (13%) of 136 bacterial infections, and other infections occurred in 52 (38% Urinary tract infections) and 24 patients (18%) (eg, osteomyelitis, peritonitis and meningitis).Conclusions. This report describes the complications and related mortality that occur soon after diagnosis of myeloma is made. Measures to prevent infectious complications has been described previously. In addition, reduction of renal toxicity also has to be mandatory.

1217

INTERNATIONAL STAGING SYSTEM FOR MULTIPLE MYELOMA IN A MEXICAN POPULATION

V. Jimenez

INCMNSZ, MEXICO CITY, Mexico

Background. Studies conducted in the 1960´s and early 1970`s identified a number of clinical and laboratory parameters that are independent predictors of survival duration. In 1975, Durie and Salmon introduced a staging system that predicted myeloma cell tumor burden. Factors in the DS classification included the level and type of monoclonal protein, hemoglobin, calcium level, and number of bone lesions. Creatinine level (Substage A: seum creatinine <2 mg/dl; and substage B : serum creatinine > 2 mg/dL) further defined lower versus higher risk patients in each tumor mass stages. Recently Greipp et al, reported the International Staging System (ISS) as a simple and reliable staging system for multiple myeloma. The ISS system was further validated by demonstrating effectiveness in patients in North America, Europe and Asia but not in Central America. Aims. The main objective of our study was to assess the effectiveness of ISS in a mecxican population. We also reported the outcomes in terms of overall response according to each group of treatment and ISS was compared with the DS staging system.

Patients, material and methods. We enrolled all patients who fulfilled entire criteria for multiple myeloma between January 1995 and December 2005. The present study is a retrospective, descriptive , longitudinal and observational one. All patients had survival status and date of last follow-up recorded within 6 months of the data analysis. At the time of analysis, 54.1% of patients had died. Statistical analysis.Univariate and multivariate survival analysis. The variables are ranked by hazard ratio, with all being significant at the *p*<-0.001 level.Fisher's Exact test was used to evaluate the statistical significance of associations in two-way contingency tables. A p value <0.05 was considered as statistically significant. Results. One hundred and twenty two newly diagnosed multiple myeloma patients were evaluated , 76 (62.3%) male and 46 (37.7%) female, aged 46-85 (median 64). Compared with the DS classification, ISS provides a simple reliable classification of patients. ISS stage I is underrepresented, maybe because stage I patients usually are asymptomatic or not included in clinical trials. B2 microglobulin greater than 5.5 mg/dl appeared to be the most highly statistically significant result (p 0.0003) Median survivals were as follows: I , 74 months; II, 43 months and 3, 20 months (p 0.0002 for differences). We also evaluated outcomes in terms of survival; creatinine > 2 mg/dL, *p*0.03 , platelet count less than 130,000, *p*0.005 , CRP >6 mg/L p0.003 , albumin < 35 g /L (*p* 0.005) and cytogenetics abnormalities such as del 13 , resulted in worst overall survival We reported an increased mortality in those patients with 14q32 abnormalities or deletion 13 (p 0.05).Conclusion. We found that B2 microglobulin higher than 5.5 mg/ L is the best cutt off to discriminate survival in newly diagnosed MM patients. Based on this result the following question is mandatory; why are serum B2MG and serum albumin such powerful prognostic factors? Serum B2MG reflects not only tumor mass and renal function but also other as yet unknown parameters, possibly including immune function.

1218

DIFFERENCE BETWEEN MALE AND FEMALE PATIENTS WITH MULTIPLE MYELOMA ON LIPID PROFILE

A. Paximads,[1] N. Pagoni,[2] V. Papalimneou,[1] E. Kalokerinos,[2] G. Rousodimos,[1] G. Parvulescou,[1] S. Sakarelou[1]

[1]*General Hospital of Athens, ELPIS, ATHENS, Greece;* [2]*General Hospital of Athens G.Gennimatas, ATHENS, Greece*

Purpose: The aim of this study was to investigate the difference between male and female patients with multiple myeloma on lipid profile. *Material and Methods.* 77 inpatients with multiple myeloma, aged 73±7, 42 females (F) and 35 males (M) were studied. Serum protein electrophoresis and immunoelectrophoresis (IgG, IgA, IgM, and IgE) and plasma lipid levels as Total Cholesterol (TC), Triglycerides (TG), HDL and LDL, from all patients were measured and compared between females and males. All patients belong to our Internal Medicine Clinic and results were analyzed in the same laboratory. *Results.* Electrophoresis (%): Albumin 43,6±9,5, 50,6±6,2(M) - 39,8±9,1(F), *p*=0,21, β-1 globulin 3,3±1,2, 3,7±1,3(M) - 3,2±1,2(F), *p*=0,62, β-2 globulin 10,7±4,3, 14,2±2,9(M) - 8,8±3,7(F), *p*=0,57, β globulin 13,1±8,3, 10,6±3,6(M) - 14,4±9,8(F), *p*=0,16, β globulin 29,8±16,4, 21,6±11,6(M) - 34,3±17,3(F), *p*=0,27, Immunoelectrophoresis (mg/dl): IgG 2439±2636, 2999±3195(M) - 2103±2260(F), *p*=0,04, IgA 998±1489, 483±784(M) - 1308±1730(F), *p*= 0,003, IgM 59±66, 56±71(M) - 60±65(F), *p*=0,63, IgE 284±674, 61±26(M) - 507±962(F), *p*=0,02, Light chains (g/L): Ig/L _ 8,3±10,0, 12,1±10,8(M) - 1,3±1,3(F), *p*=0,004, Ig/L _ 2,9±3,7, 1,2±2,2(M) - 5,9±4,3(F), *p*=0,02, Lipid profile (mg/dl): TC 173±54, 147±81(M) - 183±35(F), *p*=0,000, TG 154±69, 108±62(M) - 175±62(F), *p*=0,64, HDL 42±12, 35±17(M) - 45±9(F), *p*=0,01 and LDL 93±42, 75±57(M) - 102±32(F), *p*=0,02. *Conclusion*: The study shows that in patients with multiple myeloma the TC, HDL and LDL are increased statistically significant in females, while the level TG between female and male have not statistically significant.

1219

KINETICS OF TUMOUR KILL DURING INDUCTION CHEMOTHERAPY FOR MULTIPLE MYELOMA USING FREQUENT FREE LIGHT CHAIN MEASUREMENTS

P. Mead,[1] C. Beardsmore,[2] S. Reid,[1] J. Hattersley,[3] P Moss,[5] G. Pratt,[4] A. Macwhannel,[2] A. Jacob,[2] S. Handa,[2] C. Craddock,[5] M. Cook,[5] S. Basu[2]

[1]*The Binding Site, BIRMINGHAM, United Kingdom;* [2]*Royal Wolverhampton Hospitals, WOLVERHAMPTON, United Kingdom;* [3]*University of Warwick, WARWICK, United Kingdom;* [4]*Birmingham Heartlands Hospital, BIRMINGHAM, United Kingdom;* [5]*University Hospital Birmingham, BIRMINGHAM, United Kingdom*

Background and Aims. Accurate monitoring of clinical response to treatment in patients with multiple myeloma is essential, if drug and transplant therapies are to be used in a timely manner. Assessment of response by measuring changes in intact immunoglobulin monoclonal proteins is inaccurate because of their long half life. In contrast, the short serum half-life of free light chains (FLC; 2-6 hrs) may allow response to chemotherapy to be assessed more rapidly. In this study we have measured changes in FLC in patients with newly diagnosed myeloma receiving induction chemotherapy, in order to determine whether they provide early measures of clinical response. *Methods*. Serum FLC levels were measured in 15 patients with newly diagnosed MM undergoing induction chemotherapy . Measurements were made at days 0,4,8,21, and then monthly (after the start of treatment). The change in the subtracted value, tumour FLC minus non-tumour FLC, was used as a measure of response. Three basic chemotherapy regimens (with some variations) were utilised: Vincristine, doxorubicin plus dexamethasone (VAD; n=5), melphalan and prednisone (MP; n=5) and cyclophosphamide, thalidomide plus dexamethasone (CTD; n=.5). Where the FLC results showed adequate response to treatment, exponential curve fitting was employed to derive the t1/2 for tumour kill. Clinical assessments (including bone marrows) at 3 months were compared with the FLC measurements. *Results*. 8 patients had sufficient response for t1/2 calculation; 1 of these was from the MP treatment group (t1/2 = 18 days) and the remaining 7 from the dexamethasone based regimens (t1/2 = 5 days). At 3 months, 6/15 patients had died or had disease progression and their mean FLC fall at 1 month, was 30% (only 1 was >50%). Patients with minimal/partial clinical response at 3 months had a mean FLC fall of 57% at 1 month while the 2 patients with very good partial or complete clinical response showed FLC falls of 98% and 88%, respectively, after 1 month. 10/15 patients showed transient FLC falls (between days 4 and 21) possibly due to temporary oedema. One patient had repeated FLC falls and recoveries after each cycle of treatment probably reflecting tumour kill and regrowth. Another showed a significant fall in their IgA kappa monoclonal protein (13.9-3.6 g/L) at 1 month with only 26% fall in free kappa over the same period (discordant response). This could have been due to an unrecognised biclonal tumor, similar to other reports of FLC *breakthrough* at relapse of IgA tumours. Their final clinical response at 3 months was minimal and they died shortly afterwards. *Conclusions*. Patients showed slower FLC reductions with MP treatment than with regimens containing dexamethasone. The extent of SFLC response was generally predictive of the final clinical assessment at 3 months and this may help to separate non responders from good responders as early as within 1 month of starting therapy. This may represent an important observation in the treatment of myeloma and is now being assessed in a larger cohort study.

1220

VELCADE AN ACTIVE AGENT FOR MULTIPLE MYELOMA PATIENTS. EXPERIENCE OF A SINGLE CENTER

M.T. Petrucci,[1] C. Gallucci,[2] V. Martini,[2] A. Levi,[2] P. Del Bianco,[2] R. Foà[2]

[1]*University La Sapienza Roma, ROMA, Italy;* [2]*Haematology, University La Sapienza, ROME, Italy*

Velcade (PS-341, Bortezemib), a proteasome inhibitor, has been shown to be efficient in the treatment of relapsed and refractory Multiple Myeloma (MM) and approved for this indication. Aims. We report the results of a nearly 3-year experience regarding the use of this drug in this subset of patients followed in our center. Between April 2003 and February 2006 we treated 27 patients with MM. Patient population consists of 15 males and 12 females, with a median age of 55 years (range 32-76), 17 were IgG, 9 IgA, 1 light chain. All patients were in stage III of disease with a median time of observation (from diagnosis

to Velcade therapy) of 54 months (range 11-120), pretreated at least with two lines of therapies and were refractory or in relapse after the last treatment. Thirteen patients had underwent autologous transplantation and 2 allogeneic one, with a median number of previous therapeutic lines of 2 (range 1-5). Velcade 1.3 mg/m^2 was administered on day 1, 4, 8 and 11 of a 21-day treatment cycle for 8 cycles according to the tolerability and response in day hospital regime. A median of 6 cycles (range 2-8) were administered to the overall population. Thirteen patients concluded their program and 14 discontinued the treatment: 1 because received allogenic stem cell transplantation, 8 for adverse events and 5 for progression of disease. In this heavily pretreated population our primary end point was to obtain a decline in Monoclonal Component (MC) of at least 25%. All patients but 2 were considerate evaluable for response because treated at least with 3 cycles of therapy. Thirteen patients responded to treatment: 7 (28%) achieved a reduction of MC level > 75%, 4 (16%) < 75% and > 50% and 2 (8%) < 50% and > 25%. Twelve (48%) showed no response. The median number of cycles to achieve a response was 3 (range 1-8). After a median time of observation of 28 months (range 5-34) the median duration of response was 7.5 months with 5 patients still in response, 8 relapsed and 4 of them died for progression of disease. Among the 12 (48%) not responding patients 3 died. The majority of adverse events, resolved with the discontinuation of treatment, were nausea, vomiting, diarrhea, fatigue, thrombocytopenia and peripheral neuropathy. Taking into consideration the very poor prognosis of our patients, this study adds further evidence concerning the efficacy of this new drug. Velcade can be considerate an effective anti-myeloma drug even though its toxicity must be taken into account in designing new clinical trials.

1221

BORTEZOMIB WITH OR WITHOUT DEXAMETHASONE IN HEAVILY PRETREATED MYELOMA PATIENTS : PRELIMINARY SAFETY AND ACTIVITY PROFILE FROM A SINGLE CENTRE

A. Vassou, M. Stoura, N. Georgiannos, G. Aretos, E. Hatzimichail, K.L. Bourantas

Ioannina University Hospital, IOANNINA, Greece

Background/Aims. Patients with multiple myeloma respond to front-line chemotherapy but relapse is virtually inevitable and response duration decreases with each salvage regimen. Bortezomib, a proteasome inhibitor, is approved for the treatment of relapsed myeloma, while addition of dexamethasone may result in enhanced tumour control. We evaluated the activity and safety of bortezomib with or without dexamethasone in 20 pretreated myeloma patients *Methods*. 20 heavily pretreated myeloma patients (median number of previous therapies 4), with a median age of 73 years (range 54- 82), received bortezomib 1.3 mg/m^2 intravenously on days 1, 4, 8 and 11 of a 21-day cycle for eight cycles. When it was combined with dexamethasone, this was given on days 1,2 of each cycle at dose 20mg daily. Bortezomib was withheld if grade 3 toxicity occurred, the dose reduced to 1 mg/m^2 or 0.7mg/m^2 in the event of drug-related grade 3 non-haematological toxicity (WHO toxicity criteria). Response evaluation according to EBMT criteria was performed every two cycles. 12/20 patients received the combination of bortezomib with dexamethasone and 8 bortezomib monotherapy when contraindication for steroid administration existed. *Results*. No patient received eight cycles of treatment due to toxicity. The median number of cycles administred was 4 (range 1-6). The toxic event most frequently responsible for therapy withdrawal was grade 3 peripheral neuropathy. Thirteen cases of grade 3 peripheral neuropathy were observed. Thrombocytopenia was the most frequent adverse event (3 cases of grade 4 and 9 of grade 3) but no severe hemorrhagic episode took place. Three patients had an episode of paralytic ileus leading to treatment discontinuation.With a median follow-up of 11 months, 17 patients had a response (1CR - 16 PR), while three patients had refractory disease. 14 of 20 patients are alive and 7 out of 20 in remission. The median time to progression was 12 months and the 1-year progression free survival was 54% (8.4-15.6 months). For the monotherapy group the median time to progression was 10 months whereas for the combination dexamethasone - bortezomib 12 months, a difference not siginificant (Log rank 2-sided p=0.72). Among responders the median duration of response was 7 months (range 3-12). For the monotherapy and combined treatment groups the median duration of response was 5 and 7.5 months respectively (Student t-test p=0.65). The 1-year overall survival is 60% with the median overall survival not reached yet. *Conclusion*. We report evidence of satisfactory activity of bortezomib/dexamethasone in this group of 20 heavily pretreated patients with advanced myeloma. Peripheral neuropathy was expectedly a major problem in a patient cohort pretreated with nerve-damaging therapies such as VAD and thalidomide. Neurologic toxicity caused reduction of dose-intensity and bortezomib discontinuation, factors abrogating the overall antitumour effect. Research efforts towards modulation of neurotoxicity and optimisation of bortezomib schedules may pave the way for enhanced myeloma control.

1222

LEUKOCYTE ALKALINE PHOSPHATASE SCORE IN MULTIPLE MYELOMA; CORRELATION WITH G-CSF , IL-6 AND TNF-α

T. Kurti, T. Caja, P. Xhumari

University Hospital Center Tirana, TIRANA, Albania

Backgrounds. The leukocyte alkaline phosphatase (LAP) score has been reported to be elevated in patients with multuiple myeloma (MM), but its clinical significance has not been clarified. Some authors reported that interleukin-1α,IL-6 and G-CSF genes were co-expressed in most patients with MM. *Aim*: In the present study ,we determined the LAP scores of peripheral blood neutrophils and the serum levels of G-CSF,IL-6 and tumor necrosis factor-α (TNF-α) in patients with MM(at diagnosis), and healthy controls and made the respective correlations. *Material and Methods.* We examined 25 patients with MM, age 62±10, at diagnosis and 10 normal subjects, age 45±5. The LAP score was examined by using naphthol AS-BI phosphate (Sigma Chemical St.Luis,MO).The serum levels of G-CSF,IL-6 and TNF-α were measured by a sandwich enzyme immunoassay test (R&D Sistems,Minneapolis, MN, USa) and measured in a Microplate Reader Sirio-Brio™ (Radim Group). *Results.* The mean LAP scores of patients with MM vs control subjects were 295±58 and 187±46 respectively.The mean value of LAP in MM patients is significantly higher (p<0.001).The serum G-CSF levels of patients with MM vs those of controls were 15.2±12.4 pg/mL and 3.8±2.9pg/ml.The mean value of G-CSF in MM patients was significantly higher than the control group (p<0.01).The serum levels of IL-6 in MM patients was 6.7±13.2pg/ml, while in the control group it was under the minimal detectable level.The serum TNF-α levels of the patients with MM were 3.9±6.8pg/ml vs 0.08±0.18 pg/ml of the control subjects, showing a significant higher mean level in the MM patients vs the normal subjects (p<0.05) The correlation coefficients between the LAP score and the serum levels of G-CSF ,IL-6 and TNF-α were 0.450 (p<0.001), 0.270 (p<0.05) and 0.330 (p<0.01). *Conclusion*. The LAP score and the concentrations of serum cytokines : IL-6,TNF-α and G-CSF were higher in MM patients vs normal subjects. The most significant correlation was noted between the LAP scores and the G-CSF level. This finding suggests that the increase of the LAP score in MM may reflect a stimulation of the neutrophils by G-CSF.

1223

PROGNOSTIC FACTORS AFTER FIRST COURSE VAD (VINCRISTINE, DOXORUBICIN, DEXAMETHASONE) IN MULTIPLE MYELOMA PATIENTS TREATING FOLLOWING ASCT

G.C. Charlinski, E. Wiater, J. Dwilewicz-Trojaczek

Medical University, WARSAW, Portugal

Backgrounds. High-dose melphalan with peripheral blood stem cell rescue represents today the standard therapy for young multiple myeloma (MM) patients. The most frequent therapy inducing remission is chemotherapy according to VAD protocol. *Aims.* The aim of this study was to determine prognostic factors and overal survival (OS) according to results of first VAD chemotherapy. *Materials and metods*: The study group consisted of 64 MM patients (32M/32F), median age 57.5 y; range 35-75yr. Diagnosis was established on the base of common rules. Patients were in the stage of the clinical progression of the disease on base of Durie-Salmon scale: 9 pts at I, 15-II, 40-III. The appearance of monoclonal protein IgG class was observed in the serum at 36 cases of the patients, IgA-11, IgD-1, Bence Jones-15 and nonsecretory MM-1. Patients achieved following types of chemotherapy: 64 pts received chemotherapy according to VAD with following auto-PBSCT. We divided pts in 2 groups: First group: Pts, who are died; OS <740 days (median OS-740 days), second group: Pts, who are living; OS >740 days. We compared results of investigations/studies, wchich are made to recognise MM before and after first VAD course. All of the results have been statistically tested by using T-student test for the independent groups. For statistically significant results were p<0.05. *Results.* The baseline serum concentration of creatinine was statistically significant (p=0.05) larger in first group (mean: 4.6 mg/dl +4.17; range: 0.59-14.36 mg/dL) according to the second one (mean: 1.01 mg/dL +2.34; range: 0.66-11.3 mg/dL). We detected statistically significant results (p<0.05): -decrease in baseline concentration of monoclonal protein (mean): in first group from 5.5 g/dL (range: 0.36-13.85 g/dL +3.8) to 4.4 g/dL (range: 0.6-10.3 g/dL +3.1); in

second one (mean) from 4.7 g/dL (range: 0.6-9.3 g/dL +2.5) to 3.0 g/L (range: 0.6-9.1 g/dL +2.2); reduction of β2-microglobulin in the second group from mean: 8 mg/L (range: 0.46-67.7 mg/L +13.65) to 6 mg/L (range: 0.98-67.7 mg/L +13.28); differences in: baseline 24 hour urine calcium between groups; mean: 3.24 mmol (range: 0.16-18.5 mmol +3.8)-in first group according to: 6.5 mmol (range: 0.14-31.5 mmol +7.8); value of decrease of 24 hour urine calcium between groups: mean: 1.0 mmol (range: 0.02-2.19 mmol +0.08)-in first group, mean-4.88 mmol (range: 0-31.33 mmol +8.6)-in second one; an increase in 24 hour urine protein in first group (mean): from 2.71 g (range: 0.03-8.2 +2.44) to 4.32g (range: 0.15-20.14; +6.8) and a decrease (mean)-in second group: from 1.3g (range: 0-9 +2.2) to 0.23g (range: 0-1.4g +0.3); difference in 24 hour urine protein after first VAD chemotherapy between first and second groups: 4.32g vs 0.23g; differences between first and second groups in (mean): time to progression: 43 vs 380 days; time to new chemotherapy: 52 vs 475 days; OS: 300 vs 1145 days. *Conclusion*. Prognostic factors, which are determined OS in pts receiving VAD chemotherapy can be: 1 Reduction of monoclonal protein after first VAD course. 2 Reduction of β2-microglobulin after first VAD course. 3. Decrease in 24 hour urine protein and calcium after first VAD chemotherapy.

1224

RENAL SAFETY AND PHARMACOKINETICS OF IBANDRONATE IN MULTIPLE MYELOMA PATIENTS WITH PRE-EXISTING RENAL INSUFFICIENCY

R. Bergner,[1] B. Nauth,[2] D.M. Henrich,[1] M. Hoffmann,[3] M. Ullmann,[4] A. Honecker,[5] D. Nagel,[5] M. Uppenkamp[2]

[1]*Medizinische Klinik A, LUDWIGSHAFEN AM RHEIN, Germany;* [2]*Klinikum der Stadt Ludwigshafen gGmbH, LUDWIGSHAFEN AM RHEIN, Germany;* [3]*Oberarzt der Medizinischen Klinik A, LUDWIGSHAFEN, Germany;* [4]*MDS-Pharma Services, ZURICH, Switzerland;* [5]*Institut fr Klinische Chemie, LUDWIGSHAFEN AM RHEIN, Germany*

Backgrounds. As multiple myeloma progresses, patients are at increasing risk of skeletal complications and renal deterioration. Bisphosphonates are the standard of care for the treatment of skeletal complications due to bone metastases from multiple myeloma and other malignancies. Clinical studies have shown that some intravenous bisphosphonates are associated with an increased risk of renal toxicity. The aminobisphosphonate, ibandronate, is indicated for use in patients with bone metastases from breast cancer. In phase III trials, intravenous ibandronate had a renal safety profile comparable to placebo. *Aims.* In this open-label study we assessed the pharmacokinetics and safety of intravenous ibandronate 6mg in patients (n=40) with multiple myeloma and pre-existing renal insufficiency. *Methods.* Renal function deterioration was graded at baseline depending on creatinine clearance (grade 0: >80, 1: 50'79, 2: 30'49, 3: <30 mL/min). Patients received intravenous ibandronate 6mg (30 minute infusion). Ibandronate excretion and serum levels were measured over 24 hours. To minimize error, creatinine clearance was calculated using three methods (direct serum/urine measurements, Cockcroft and Gault [*Nephron 1976;16:31-41*] and Modification of Diet in Renal Disease Study Group [*MDRD; Ann Intern Med 1999;130:461-70*]). AUC of serum ibandronate levels and ibandronate clearance were calculated. Markers of tubular damage, β-glutathione-S-transferase [βGST] and α-N-acetyl-glucosaminidase [βNAG], were measured at baseline, and at 24 and 72 hours following ibandronate infusion. *Results.* At baseline ten patients had normal renal function (stage 0). The remaining thirty patients had varying degrees of renal insufficiency, with ten evaluated as stage 3. There was a positive correlation between ibandronate elimination and creatinine clearance (r=0.87; $p<0.00001$). Total body clearance of ibandronate did not change significantly with renal insufficiency. The AUC for stage 3 renal insufficiency increased by ~50% versus stage 0 ($p<0.02$). The AUC for ibandronate was not significantly different between other grades of renal function. Serum creatinine and urinary enzymes of tubular damage did not change significantly within 72 hours of ibandronate infusion. No acute nephrotoxicity was seen throughout the study. *Summary and Conclusions.* In this study, the elimination of ibandronate correlated with renal function. The exposure (AUC) of ibandronate serum levels significantly increased for patients with the most advanced renal deterioration. Due to its renal safety profile in phase III clinical trials, ibandronate is indicated in patients with mild-to-moderate renal impairment without dose adjustment. Ibandronate is the only bisphosphonate that is recommended for use in patients with severe (grade 3) renal insufficiency (ibandronate 2 mg). We conclude that a dose reduction is not necessary to maintain renal health. Despite renal function already being compromised in this patient group there was no evidence of acute nephrotoxicity with ibandronate. These data suggest that ibandronate may be suitable for use in multiple myeloma patients with or without pre-existing renal impairment without the need to reduce the dosage.

1225

RISES IN SERUM ALKALINE PHOSPHATASE (AP) ARE NOT CORRELATED WITH RESPONSE TO VELCADE (BORTEZOMIB)

M. H. van Droogenbroeck,[1] D. Selleslag,[1] K. Van Eygen,[2] K. Geldhof,[3] F. Nollet,[1] M. Hidajat,[1] J. Billiet,[1] A. Criel[1], A. Van Hoof[1]

[1]*AZ St Jan AV, BRUGGE, Belgium;* [2]*AZ Groeninge, KORTRIJK, Belgium;* [3]*Yperman ZH, IEPER, Belgium*

Backgrounds. Velcade, a proteasome inhibitor, is a novel agent in the treatment of multiple myeloma (MM), showing promising activity even in relapsed/refractory MM. Recently Zangari and collaborators repeatedly claimed a clear correlation between rise in serum AP and activity of Velcade (abstract Sydney '05, oral presentation ASH '05 and Brit J of Haematol '05). Serum AP could be a cheap and easy test to decide on an expensive treatment. We have/had a different impression and studied our myeloma population treated with Velcade, partly in a retrospective, partly in a prospective way. *Methods.* Between March '03 and August '05 32 evaluable patients were treated with Velcade for relapsed/refractory MM at our institution. 4 Patients presented with a light chain λ, 3 with light chain κ, 3 with IgA κ, 2 with IgAλ, 12 with IgGκ, 7 with IgG λ, and finally 1 with a non secreting MM. The youngest patient was 53 years, the oldest 85; 19 were male, 13 female. Prior to Velcade, they were treated with a mean of 3 lines of therapy (1-8); 16 underwent at least one stem cell transplantation. *Results.* The best responses to bortezomib were: 5 nCR, 14 PR, 3 MR, 4 SD and 6 PD (EBMT response criteria). We checked serum AP at baseline and after 6 weeks (strongest predictive value in the study of Zangari). We detected a rise in AP (8-41%) in all groups of response - even in progressive disease - indeed suggesting an osteoblastic activity, but no correlation with the type of response. *Conclusions.* 1. Velcade probably has an osteoblastic activity (besides other mechanisms of action), reflected in rises in AP. 2. In contradiction with a recent publication of Zangari *et al.*, there is no correlation between myeloma response and level of AP increase.

1226

A SINGLE FIXED DOSE OF PEG-FILGRASTIM ALLOWS ADEQUATE STEM CELL MOBILISATION IN MULTIPLE MYELOMA PATIENTS

P. Zappasodi,[1] A. Corso,[1] T. Habermann,[2] C. Barat,[3] C. Astori[1], M. Bonfichi,[1] M. Varettoni,[1] S. Mangiacavalli,[1] E. Morra,[3] M. Lazzarino[1]

[1]*IRCCS Policlinico S. Matteo, University, PAVIA, Italy;* [2]*Mayo Clinic College of Medicine, ROCHESTER, MN, USA;* [3]*Division of Hematology, Ca' Granda Niguarda, MILAN, Italy*

Backgrounds. Autologous transplant is the standard of care for multiple myeloma (MM) patients aged less than 65 years. An adequate mobilization of stem cells is therefore essential to complete the therapeutic program. PEG-filgrastim (PEGf), a long lasting conjugated form of filgrastim used as a single dose of 6 mg to shorten chemotherapy induced neutropenia, has been recently evaluated for stem cells mobilization in haematologic malignancies. Preliminary reports show that PEGf is as effective as filgrastim in mobilizing MM patients with a better compliance. *Aims.* To evaluate the mobilization capacity and the safety of PEGf after DCEP regimen in MM patients enrolled in a high dose program. *Methods.* We mobilized 11 previously untreated MM patients with a combination of DCEP chemotherapy (Decadron 40 mg/day i.v. days 1-4, Cyclophosphamide 700 mg/m^2/day i.v. days 1-2, Etoposide 100 mg/sqm/day i.v. days 1-2, Cis-Platin 25 mg/m^2/day i.v. days 1-2) followed by a single subcutaneous dose of PEGf 6 mg 48 hours after the end of chemotherapy. The first leukapheresis was performed when peripheral CD34$^+$ cells were >20/μl and continued until at least 4×10^6/kg CD34$^+$ cells were collected. Patients collecting <2×10^6/kg CD34$^+$ cells were considered poor mobilisers. *Results.* The median number of CD34$^+$ cells collected with 1 (6 patients) or 2 leucapheresis (5 patients) was 5.9×10^6/kg (range: 1.5-29.4). Nine patients mobilized 10 days after the end of DCEP therapy, 1 patient after 9 days, 1 patient after 11 days. One patient who had failed the first mobilization with filgrastim, with PEGf collected 3.5×10^6/kg CD34+ cells in two leukaphereses. One patient did not mobilise (1/11: 9%). Median peak number of peripheral CD34$^+$ cells at the time of collection was 60/mL (range: 24-418). Five patients showed WHO grade 3-4 therapy-related neutropenia and 2 WHO grade 2 throm-

bocytopenia. No patient experienced fever or infections or required transfusions. The majority of the patients complained of mild to moderate back pain easily controlled by oral analgesics. *Conclusions.* This study shows that a single fixed dose (6 mg s.c.) of PEGf is safe and effective to mobilize adequate number of CD34+ cells in the majority of myeloma patients, a category usually considered worse mobiliser than patients with other hematological malignancies. In addition, the single administration of PEGf shows better compliance than repetitive doses of filgrastim.

1227

EXANTHEMA AND HERPES ZOSTER INFECTION DURING VELCADE USE INCIDENCE, TREATMENT AND PROFYLAXIS

L.P. Pour, R.H. Hajek, Z.A. Adam, M.K. Krejci, A.K. Krivanova, Z.L. Zahradova, T.B. Buchler, J. Vorlicek

Faculty hospital Brno, BRNO, Czech Republic

Backgrounds. Bortezomib has been shown to be highly effective in the treatment of relapsed multiple myeloma (MM) . Data from clinical trials show that the incidence of herpes zoster during bortezomib therapy is about 13%. Skin rash is quite common toxicity seen in MM patients treated with bortezomib. Where reported, its incidence in clinical trials ranged from 8 to 18%. We reported our results and treatment of this two adverse effects of bortezomib treatment. *Methods.* From December 2004 we treated 48 relapsed MM patients with bortezomib. Patients were treated with standard dosage schedule (intravenous infusions of bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11 of a 21-day cycle). *Results.* Our first ten patients treated with bortezomib did not receive varicella-zoster virus (VZV) prophylaxis and herpes zoster developed in three of these patients (33%). Clinical manifestation of herpes zoster was typical, starting with itching and pain and exanthema appearing later. All patients were treated for the 2nd relapse of MM. Herpes zoster developed in the first patient during the 3rd cycle, in second patient during 6th cycle and in third patient during the 8th cycle of bortezomib. Therapy with bortezomib was subsequently interrupted and all three patients received treatment with acyclovir intravenously. Based on experience, we started to use prophylaxis with acyclovir 400 mg per os 3 times daily during bortezomib therapy. We did not note any VZV reactivations in 36 consecutive patient receiving VZV prophylaxis. This group also included five patients who already had VZV reactivation before bortezomib treatment. In 12 of 48 patients (25%), rash developed during the second treatment cycle.T he first cycle of bortezomib was well tolerated in all cases. Skin biopsy was done in first three patients , in all cases perivascular lymphoid infiltrates were found. Tash resolved rapidly in all cases after treatment with prednisone 20 mg/day Two patients were treated with prednisone and cetirizine (10 mg/day). After resolution of rash, prednisone was discontinued, but rash recurred with the next bortezomib infusions despite continued treatment with cetirizine. To prevent recurrence of the rash, it was necessary to administer corticosteroids (10 mg prednisone) prophylactically before every administration of bortezomib. Two patients with bortezomib-associated rash were treated with dexamethasone together with bortezomib from 3rd cycle onwards due to minimal treatment response. In these patients, rash resolved and did not recur. *Conclusions.* VZV reactivation is common and serious consequence of bortezomib therapy. According to our experience, prophylaxis with acyclovir is very effective and should be considered for all patients treated by bortezomib. The minimal sufficient dose of acyclovir for prophylaxis of VZV reactivation remains to be established but in our group of patients dose of 400mg of acyclovir thrice daily was effective in 100% of cases. Rash is common toxicity seen in patients treated with bortezomib. In our cases lesions are infiltrated by lymphocytes seem to be the most typical after bortezomib. According to our clinical experience, corticosteroids are useful for prevention and treatment of bortezomib-associated rash while maintenance treatment with antihistamines alone is not effective.

1228

SELECTED ELDERLY MYELOMA PATIENTS CAN BENEFIT FROM AUTOLOGOUS STEM CELL TRANSPLANTATION AND HAVE A SIMILAR CLINICAL OUTCOME AS YOUNGER PATIENTS: A SINGLE CENTRE STUDY

L. Ysebrant, N. Meuleman, J. Bennani, I. Ahmad, M.C. Ngirabacu, C. Nguyen van Cau, P. Lewalle, O. Dehenau, D. Bron

Jules Bordet, BRUSSEL, Belgium

High-dose chemotherapy with autologous stem cell transplantation (ASCT) is currently the standard treatment in myeloma patients below 65 years but transplant remains questionable in elderly patients. The aim of this study is to evaluate the feasibility and the efficacy of ASCT in elderly myeloma patients. We retrospectively reviewed the medical files of forty seven patients with stage II or III multiple myeloma treated with high doses melphalan followed by ASCT between 1995-2003.We compared the clinical outcome of patients older than 65 years at the time of the ASCT to younger ones. Progression-free survivals (PFS) and overall-survivals (OS) curves were compared using the Kaplan-Meyer model on an intent-to-treat basis. Forty seven symptomatic myeloma patients treated with ASCT were followed: 10 patients were 65 years ore more and 37 patients were younger. Median age at the time of ASCT was respectively 67 years (65-71) and 55 years (39-63).There were no significant differences in the distribution of pre-treatment characteristics: ß2 microglobulinemia, chromosome 13 deletion, renal dysfunction, stages (two patients were diagnosed stage II and forty five at stage III) , PS and number of comorbidities. Sixty eight percent of the patients had none or single comorbidity. There were no significant differences in median PFS between patients older than 65 years and younger (13 months versus 17months, p=0.36) and in median OS, respectively 57 months versus 59 months (p=0.32). A trend to a better complete remission rate was observed in younger patients (p=0.09) but good partial remission rate was similar in both groups of patients. Transplant related mortality (TRM) was 0% and serious adverse events (SAE) are similar in both groups (34% vs 36%). Clinical outcome was similar in both groups of patients treated by ASCT. OS, TRM and SAE of elderly patients (65+) were improved compared to previous studies. These differences could be explained by the population of fit elderly patients with few adverse prognosis factors (β2 microglobuline, chromosome 13 deletion, renal dysfunction) in our study. Selected elderly patients with few comorbidities, can benefit from ASCT and have a similar clinical outcome as younger patients.

1229

PLASMA CELL IMMUNOPHENOTYPE CD56 POSITIVE AS GOOD PROGNOSIS MARKER

H. Bumbea,[1] A.M. Vladareanu,[1] M. Begu,[1] I. Tuca,[1] V. Motronea Vasilache,[1] D. Casleanu,[1] I. Voican,[1] C. Marinescu,[1] C. Ciufu[1], A. Petre,[1] V. Popov[2]

[1]*Emergency Universitary Hospital, BUCHAREST, Romania;* [2]*Spitalul Judetean, PITESTI, Romania*

Background. Malignant plasma cell could have a classical appearance, or it could be atypical at the optical microscopy analysis. This is known to be a B-cell without expression of lineage markers (CD19, CD20), with lack of CD45 expression and typical expression of CD38, CD138, and CD56. There were described aberrant coexpressions of CD10, CD28, c-kit, CD20 in a few cases. *Aims*. We have analyzed our cases of multiple myeloma and plasma cell leukemia to correlate with the immunophenotype and microscopic appearance. *Methods*. We performed optical microscopy and immunophenotyping on peripheral blood cells and bone marrow aspirate, in 67 patients, with median age of 63 years. *Results*. We found on optical microscopy classical plasma cell in 84,3% and 15,7% plasmoblasts. Immunophenotyping by flowcytometry found in 70% of patients the expression of CD38, CD138 and CD56. In 15% we found lack for CD56 and in 20% lack for CD38. We found also that those patients with lack for CD56 had poor prognosis and the lack for CD38 didn't change the prognosis. In a patient with plasma cell leukemia positive expression of CD56 could be considered as good prognosis marker, despite of aberrant lack of CD38 expression on plasmoblasts. Aberrant coexpression of CD20, CD13 or CD33 didn't associate poor outcome. In 5% patients we found plasma cells in peripheral blood associated with poor prognosis and terminal phase of disease. In conclusion, we consider that immunophenotyping in plasma cell leukemia and multiple myeloma is very important, and critical for the quickly diagnosis, too. We can find important prognostic markers, and we consider that lack of expression of CD56 could be the most important, associated with poor prognosis.

1230

MORPHOFUNCTIONAL STATUS OF LIVING PLATELETS AND THROMBOSIS RISK IN PATIENTS WITH CHRONIC RENAL FAILURE AT THE END STAGE OF HEMODIALYSIS

E. Vlasova, I. Vasilenko, V. Metelin, V. Shabalin, S. Babakova

Rheumatology, MOSCOW, Russian Federation

Backgrounds. Heparin-induced thrombocytopenia and thrombosis is a severe complications in patients on hemodialysis. To appreciate the character of cellular hemostasis disorders in patients with chronic renal fail-

ure (CRH) by using criterias vital estimation of morphofunctional status of platelet peripheral blood, to determine early morphometrical markers prethrombotic status. *Methods.* 21 healthy volunteers (the control group) and 27 patients with (RH) before and after carrying out procedures of hemodialysis (HD) have been examined. Platelet-rich plasma (PRP) was separated from patient and control blood samples by centrifugation at 1000g for 10 min at room temperature. Drops of unfixed cells were placed on mirror surface and allowed to investigate for intervals of 10-15 min. Morphofunctional status of platelet peripheral blood we determined by method of vital computer morphometry with using computer phase-inteference microscope (CPM) *Cytoscan* (Moscow, Russia). The main *Cytoscan* specifications are: height accuracy 0.5 nm, coordinate accuracy 10 nm, image area from 64x64 to 256x256 pixels, optical magnification 1000, acquisition time 4-30 sec. Platelet phase images were obtained by CPM and analyzed with MATLAB version 6.5. At one time standard hemostasis studies of peripheral blood patients were performed. Results. The results of studies showed that mean optic-geometrical parameters of living platelets in the control group (diameter, perimeter, high, area and volume) have constituted 2,6±0,8 mkm; 8,2±3,4 mkm; 1,2±0,5 mkm; 4,6±2,1 mkm^2; 1,8±1,3 mkm^3 (Mo). We have analyzed the optic-geometrical parameters of each isolated platelet and the distribution of human platelets by sizes to detect the heterogeneity of cell population. It allowed to identify four platelet forms that have different morphological features and different parameters of size distribution. In the cell population we distinguished 4 morphologic forms of platelet with according to various activation levels: 62% of resting platelets (discoid), 21% of platelets with low activation level, 12% of platelets with high activation level and 5% of degenerate functionally incomplete platelets. In CRF mean metric platelets parameters were constituted 3,1 ±1,0 mkm; 9,4±3,1 mkm; 1,0±0,3 mkm; 5,9±1,9 mkm^2; 2,3±1,1 mkm^3 (p<0,05). The proportion of different morphological cell types were 43%; 44%; 11%; 2%, respectively. The individual features of platelets reactivity of peripheral blood in patients with CRF (measure metric parameters and morphologic platelet reconstruction) after the standard procedure of hemodialysis have been educed. Conclusions. The computer morphometry of living platelets is guarantied rapid and objective analysis of the platelet hemostasis, showing early appearances of platelet complications in patients with CRF. Moreover morphometric parameters of living platelets can be predictors of possible following hemostasiological disorders in recipients of renal graft during early postoperative time. The proposed method opens additional vistas for analyzing the heterogeneity of platelet populations without sophisticating experimental techniques.

1231

HELICOBACTER PYLORI ERADICATION IN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

M.Y. Yilmaz, V.O. Okan, M.P. Pehlivan, Y.P. Pehlivan, C.K. Kis

Gaziantep University, GAZIANTEP, Turkey

Idiopathic thrombocytopenic purpura (ITP) is autoimmune disease which autoantibodies are responsible for the mechanism of platelet destruction. Some studies have reported the presence of Helicobacter pylori infection with autoimmune disease particularly with ITP. There are increasing findings with the association between eradication of H.pylori infection and significant increase in platelet count. Aims. The primary aim of our study to asses platelet count increase after H.pylori eradication therapy in 7 ITP patients. Methods. In the present study we describe 7 ITP patients which H.pylori was diagnosed. This prospective study was carried out in the department of hematology of Gaziantep University between May 2004 and February 2006. There were1 male, 6 female with a median age of 29 (range 20-48). Mean platelet count was 49.000/µL. Patients who were known to have any chronic or systemic disease, considered at risk for bleeding or platelet count lower than 10.000/µL and those younger than 16 years of age were excluded. Diagnosis of ITP was established if patient had platelet count <100.000/µL for 6 months, exclusion of the other causes of thrombocytopenia and normal or megakaryocytic hyperplasia of bone marrow aspiration. H.pylori infection was assessed by means of a 14C-urea breath test and positivity was defined as positive result on urea breath test. All patients recieved the anti-*H.pylori* infection eradication tripple therapy (amoxicilline 750mgr b.i.d, claritromycine 400 mgr b.i.d, lansoprozole 30 mgr b.i.d). Platelet number was monitored every two weeks until six months after H.pylori eradication therapy. In 7 patients 14C-urea breath test were positive. Megakarycytic hyperplasia was seen in bone marrow 4 of these patients. *Results*. Three of seven patients platelet count increased at least 30.000/µL of baseline value (median 60000//µL) /µL with H.pylori eradication therapy (%42). Four was accepted as non-responder of H.pylori eradiction therapy. Two patient of them had been treated with steroids and the other two patients platelet counts are 65.000/µL and 54.000/µL respectively. Conclusions. Eradication therapy of H.pylori infection is effective in ITP treatment. Even though the pathogenetic mechanisms of *H.pylori* dependent thrombcytopenia remain obscure, when *H.pylori* was established eradication therapy should be started in ITP patients. But further studies on large number of patients are needed.

1232

DETERMINATION OF THE RELATIONSHIP BETWEEN PLASMA HEPARIN LEVEL AND APTT AND ASCERTAINMENT OF THE APTT RANGE TO BE AIMED DURING TREATMENT OF VENOUS THROMBOEMBOLISM BY HEPARIN

E. Koca, Y. Buyukasik, D. Cetiner, I.C. Haznedaroglu, S. Aksu, H. Goker, N. Sayinalp, S. Kirazli, O.I. Ozcebe

Hacettepe University Medical School, ANKARA, Turkey

Heparin is a potent anticoagulant used in acute venous or arterial thromboembolism. Unfortunately, individual differences can be seen in anticoagulant response of patients that are treated with heparin. Actvated partial thromboplastin time (APTT) is the most widely used monitoring test. The therapeutic range for APTT can be different such as 1.5-2.5, 1.5-2, 1.8-3 times of the normal laboratory mean in the literature. APTT is not correlated with blood heparin consentration or its antithrombotic effect. Different APTT reagents may have different responses to heparin. This may be the cause of the difference between the therapuetic ranges suggested in the literature. When we consider all of these data, making therapeutic range calibration for each APTT reagent corresponding to heparin levels 0.2 - 0.4 U/ml by prothamine sulphate titration or anti-Xa level of 0.3 - 0.7 U/ml may be an appropriate approach. Using anti-Xa assay is cheaper and easier than prothamine sulphate titration. The aim of this study is to determine the therapuetic APTT range by using anti-Xa assay and whether there is a difference between these old and new ranges. Besides, a poll is applied among doctors working in different wards of the hospital to understand that these two therapeutic ranges ara different from the daily practice. APTT (STA CK Prest 5; Diagnostica Stago, France) and anti-Xa (STA-Rotachrom_ Heparin; Daignostica Stago, Fransa) are studied in plasma samples of patients receiving heparin hospitalised in Internal Medicine and Neurology wards because of venous thromboembolism (VTE) or serebrovascular accident (SVA) between September 2002 and June 2003. The correlation between APTT and anti-Xa was analyzed by two variant correlation analysis and linear regression analysis. There was a very good correlation (r=0.73, p<0.001). The formulation of the correlation was as follow: APTT= 37 + (68.8xAntiXa). The therapeutic APTT range calculated by using 0.3 and 0.7 U/ml anti-Xa levels were 58-85 seconds. 1.5 - 2.5 times of these values were corresponding to 47.4 - 79 seconds. When a poll was made among 22 doctors treating VTE or SVA, it was seen that the theurapeutic ranges used were different individually and from the values found in the study.

1233

PLATELET AGGREGATION ABNORMALITIES IN PATIENTS WITH THROMBOSIS OR RECURRENT FETAL LOSS (A PRELIMINARY REPORT)

C. Beyan, K. Kaptan, A. Ifran

Gulhane Military Medical Academy, ANKARA, Turkey

Background. The results of recent studies suggest that platelets have a major role in arterial and venous thrombosis and recurrent fetal loss (RFL). Aims. The purpose of this study is to evaluate the platelet aggregation abnormalities in patients with arterial thromboembolic disorders (ATE), venous thromboembolism (VTE) and RFL. *Methods*. Results of three ATE (three ischemic stroke), 17 VTE (12 deep vein thrombosis/pulmonary embolism, two portal vein thrombosis, one hepatic vein thrombosis, one renal vein thrombosis and one retinal vein thrombosis) and 28 patients with RFL were compared with 59 controls in this preliminary report. Platelet aggregation was induced by adenosin diphosphate (5 µM) (ADP), collagen (0,2 mg/ml), and epinephrine (10 µM). The analyses were performed by using a Whole Blood Lumi-Aggregometer. Cases with ATE were not evaluated as a distinct group in statistical comparison because of inadequate case number. *Results*. The whole patient group (5/48 vs. 0/59; p=0,016) and the group of patients with VTE (2/17 vs. 0/59; p=0,048) have significantly higher ratio of patients with augmented response to ADP than the control group. The whole patient group (8/48 vs. 1/59; p=0,007) and the group of patients with RFL (4/28 vs. 1/59; p=0,036) have a significantly higher ratio of patients with low

response to ADP than the control group. The group of patients with VTE (3/17 vs. 1/59; p=0,033) have a significantly higher ratio of patients with low response to collagen than the control group. The whole patient group (12/48 vs. 2/59; p=0,001), the group of patients with VTE (5/17 vs. 2/59; p=0,005), and the group of patients with RFL (5/28 vs. 2/59; p=0,033) have a significantly higher ratio of patients with low response to epinephrine than the control group. Sticky platelet syndrome-like abnormality was detected in four cases (8,3%) of the patient group (one case with type I-like in RFL group, one case with type III-like in ATE group, two case with type III-like in VTE group). *Conclusions*. Although we need data which will be obtained from many more cases for a certain evaluation, we suggest that in approaching the patients with thrombosis/RFL, platelet functions screening should be applied according the results of this preliminary report.

1234

PULMONARY EMBOLISM: EPIDEMIOLOGICAL CHARACTERISTICS ACQUIRED AND INHERENT RISK FACTORS

I. Kolaitis,[1] G. Maglaras,[2] L. Dova,[3] G. Baxevanos,[3] E. Dokou,[3] Z. Metafratzi,[4] S. Constantopoulos,[2] M. Vassiliou,[2] G. Vartholomatos[3]

[1]*Ioannina University Hospital, IOANNINA, Greece;* [2]*Pneumology Clinic,University Hospital, IOANNINA, Greece;* [3]*Hematology Laboratory, IOANNINA UNIVERSITY HOSPITAL, Greece;* [4]*Radiology Department, IOANNINA, Greece*

Backgrounds. Pulmonary embolism (P.E.) is the most serious form of venous thromboembolism. *Aims.* To analyze the epidemiological characteristics and search for risk factors among cases of PE occurred in our area (North-Western Creece). *Materials and Methods.* The study group consists from 123 adults patients (mean age= 62, SD =14,8 years) presented in the emergencies with high PE probability. All patients treated in the Pneumonology Clinic during the last four years. Four patients with definite diagnosis of severe PE, with unstable cardiovascular function that treated on the Intensive Care Unit of our Hospital, are not included. The diagnosis of PE confirmed on 74 patients (74/123, 60%) which classified as idiopathic PE (IPE), 36 cases or as secondary PE (SPE), 38 cases, according to the co-existence of cancer, lymphoma, anti-phospholipid syndrome, recent trauma or operation and /or prolonged immobilization. Among the 49 patients that we did not confirm a diagnosis of PE (non-PE) only three were found with a condition that could accompany PE. During the follow-up one death occurred that attributed to secondary PE and two patients developed recurrence. *Results.* Although the women and the older presented more frequently as cases of SPE neither the sex nor the age was statistically different between the cases of IPE, SPE or non-PE. Fibrinogen and D-Dimers levels are significantly higher among PE than non-PE patients. Protein S, protein C, Antithrombin and LDH activities are similar between the IPE and SPE patients. Lupus anticoagulant occurs less frequently among IPE than SPE cases but this difference was not significant. Common thrombophilia mutations were always found in higher frequency among IPE than cases of SPE, heterozygosity for factor V Leiden (6/29, 20.7% on IPE versus 0% on 37 SPE cases) heterozygosity for prothrombin G20210A (7/29, 24.1% on IPE versus 2/35, 5.7% on SPE cases) and homozygocity for C677T of MTHFR (7/29, 24.1% on IPE versus 4/35, 11.4% on SPE cases). Among the patients with IPE the heterozygosity for prothrombin G20210A mutation is very common in comparison to normal healthy controls (O.R.= 11.6 95% C.I.=3.8 to 35.0). In a subgroup of PE patients (24/74) and non- P.E. patients (9/49) we search for six other polymorphisms of gene encoding proteins, involved in coagulation (FV H1299R, Fib G455A, FXIII Val34Leu) or fibrinolysis (PAI-1) the platelets receptor (HPA-1) and the metabolism of homocysteine (MTHFR A1278C) but we found no difference between those groups as well as between the patients and the normal healthy controls. *Summary /Conclusions.* Pulmonary embolism is a rare but serious disease that occurs in our area, mainly on the winter, with a varied crude incidence between 35 to 55 cases per 100.000 adults per year. Half of the cases of pulmonary embolism belong to secondary cases. The low mortality rate and recurrence rate of the disease must be proved in the future. Higher fibrinogen and D-Dimers levels characterize the cases of PE. Genetic mutations are significantly more frequent in the IPE group with the heterozygosity for prothrombin G20210A gene being the most striking genetic risk factor.

1235

THE USE OF D-DIMER TEST IN DIAGNOSIS OF VTE IN EMERGENCY ROOM SETTING: EXPERIENCE OF CARMEL MEDICAL CENTER

A. Cassel,[1] R. Rubinstein,[2] O. Valkovski,[3] M. David,[4] Y. Keynan,[2] A. Kotler,[3] A. Kogan,[5] M. Quitt[3]

[1]*Carmel Medical Center, HAIFA, Israel;* [2]*Internal Medicine, HAIFA, Israel;* [3]*Hematology, HAIFA, Israel;* [4]*Laboratory Medicine, HAIFA, Israel;* [5]*Emergency Medicine, HAIFA, Israel*

Venous thromboembolism (VTE) is a common clinical entity in most emergency departments and often requires extensive diagnostic analysis. DD test is recognized as a valuable tool for screening patients suspected of VTE, allowing to ascertain the absence of thrombosis . We compared several rapid methods, and have chosen VIDAS D-Dimer by bioMerieux. The goals of this study were: 1. Following up patients with normal DD levels, who were excluded from further testing, in order to test the reliability of our method of choice. 2. Evaluating the cost-effectiveness of the use of this test. 3. Evaluating whether the test is employed according to the original indications given to the medical staff. In the first phase of the evaluation we compared the results of DD tests of a random group of 60 patients suspected of VTE to the conservative way of diagnosis of VTE that is practiced in our institution. Patients were followed for 6 months to rule out recurrent events or misdiagnosis. 37% of the patients showed normal DD levels, and none of these patients was diagnosed with VTE during the follow-up. Of the patients with elevated DD levels, only 27% were independently diagnosed with VTE. These results led us to instruct the medical staff to perform the DD test only on patients accepted to the emergency department with no underlying medical problem, and who have low to moderate probability for VTE. The test is performed in Carmel Medical Center since 2003. A random sample of 120 patients were assessed whether normative results of the DD test served the clinician to rule out further tests, and what was the outcome in terms of VTE . Whether the indications for DD testing were followed was investigated in this group as well. The results of this analysis demonstrated that: 1. The DD test is very reliable as exclusion criterion, as none of the patients with normal DD levels was diagnosed with VTE; 2. The DD testing policy (only for low to moderate VTE probability) was closely followed; 3.The use of the test for screening of patients spared further testing in most patients (85%), and thus proved to be cost-effective. The feasibility and reliability of the DD test promoted the wide use of this test. During the two-year period the use is steadily increasing. Besides its benefits, excessive use of the test is a likely possibility that needs to be assessed.

1236

VENOUS THROBOEMBOLISM (VTE) IN HAEMATOLOGICAL MALIGNANCIES: A SINGLE CENTRE EXPERIENCE

V. Bongarzoni,[1] M. Cedrone,[2] A. Chierichini,[2] B. Anaclerico,[2] S. Fenu,[2] M. Bartolini,[2] P. Iacovino,[2] G. Antonucci,[2] L. Annino[2]

[1]*Azienda Ospedaliera 'S. Giovanni', ROME, Italy;* [2]*Azienda Ospedaliera 'S. Giovanni', ROME, Italy*

Backgrounds. The overall risk of VTE is increased 7 fold in cancer patients. Within cancer population the highest risk of VTE is recorded in pts with hematological disorders(OD ratio 2.8), lung and gastroenteric cancer, respectively (*Blom, 2005*). *Aims and Methods.* The aim of this retrospective study was evaluate: a) the overall incidence of VTE in our patients with haematological malignancies, b) the incidence of a thrombophilic status in these patients. Thrombophilic screening consists of Factor V Leiden and Factor II polymorphism, PC, PS and ATIII activity, LAC and ACA Ig M and Ig G assays. From January 2004 to December 2005, 259 consecutive patients- 71 Acute Leukemias (AL), 34 Hodgkin Disease (HD), 60 Non Hodgkin's Lymphomas (NHL), 64 Myelodisplasia (MDS), 30 Multiple Myeloma (MM)- entered in this study. Patients with myeloprolipherative disorders were not included since in these cases it is well known VTE represents a frequent feature of disease. 3.Results. of the 259 patients, 20 (7.7%)- 8 males, 12 females, median age 64 y (min 30-max 80 y)- developed a VTE: 11 deep vein thrombosis, 3 inferior vena cava, 6 upper right arm. VTE occurs in 6 (8.4%) AL, 4 (11.2%) HD, 7 (12%) NHL, 1 (1.5%) MDS, 2 (6.6%) MM. 15 patients had concomitant diseases: cardiac (8), renal (1), solid cancer (1), metabolic disorders (5); furthermore 6 of these had central venous catheter, 5 were confined to bed > 30days, and 8 were giving chemotherapy. As haematological disease status at time of VTE, 15 cases had active disease (5 at onset, 11 in relapse or progression) while 5 were in complete remission (CR). Thrombophilic screening was available in 16 patients. Abnormal

tests were detected in only 4 (25%) cases; ACA IgG high title (1), reduced PC activity (1), hyperhomocysteinemia (2). In all cases VTE treatment has been successfully done with subcutaneously LMWH for 4 months at least. To date 18 of 20 patients are alive: 11 in haematological CR, 7 in stable disease, 2 patients died because of progressive disease. 4. Conclusions. In our series, if small, 75% of patients who developed VTE had an active phase of haematological disease, VTE incidence was higher in lymphomas (11.6%) with respect to other malignancies. whereas abnormal thrombophilic tests were detected in only 25% of cases.The endothelial vascular damage induced by central venous catheter plus chemotherapy proved to be determinant in the development of upper right arm VTE.

1237

IS OSTEONECROSIS ASSOCIATED WITH MUTATIONS OF THE METHYLENETETRAHYDROFOLATE REDUCTASE GENE?

G. Zalavras,[1] C. Malizos,[2] L. Dova,[3] A. Zibis,[4] E. Dokou,[3]
G. Baxevanos,[3] Z. Dailiana,[4] I. Kolaitis,[3] A. Vartholomatos[3]

[1]*University of Southern California, LOS ANGELES, USA;* [2]*University H., LARISSA, Greece;* [3]*Hematology Laboratory Unit of Molecula, IOANNINA, Greece;* [4]*University H, LARISSA, Greece*

Backgrounds. Mutations of the methylenetetrahydrofolate reductase (MTHFR) gene interfere with homocysteine metabolism, and hyperhomocysteinemia is considered a risk factor for thromboembolic complications. Intravascular coagulation constitutes the major pathogenetic pathway leading to ischemic bone death (osteonecrosis) and the role of thrombophilic gene mutations is increasingly being recognized. Aims. The purpose of our study is to investigate the presence of MTHFR mutations in patients with osteonecrosis (ON) in an effort to clarify the complex pathogenesis of the disease. *Methods.* We evaluated a patient group of 48 consecutive adults with ON and a control group of 48 healthy blood donors. All controls were matched for race, age, and gender to the patients and had no history of cardiovascular disease or thromboembolic events. Genetic analysis of the MTHFR C677T and A1298C polymorphisms was carried out by allele-specific polymerase chain reaction. *Results.* Homozygosity for the MTHFR C677T mutation was present in 6.3% (3/48) of ON patients compared to 8.3% (4/48) of controls. The difference was not statistically significant, with an odds ratio of 0.7 (95% confidence interval 0.2 to 3.5). Homozygosity for the MTHFR A1298C mutation was present in 12.5% (6/48) of ON patients compared to 10.4% (5/48) of controls. The difference was again not statistically significant, with an odds ratio of 1.2 (95% confidence interval 0.3 to 4.3). *Conclusions.* Although hyperhomocysteinemia is considered a thrombophilic factor, the potential pathogenetic role of the C677T and A1298C MTHFR mutations in thromboembolic disease remains controversial. In the current report, we detected no differences in the prevalence of these mutations in patients with ON compared to controls. Intravascular coagulation in patients with ON may be mediated by other genetically determined factors.

1238

DERMATAN SULPHATE THERAPY FOR HEPARIN-INDUCED THROMBOCYTOPENIA

G.B. Cavallero,[1] A. Zecchino,[1] E. Migliore,[1] A. Cardellicchio[1],
L. Fenoglio,[1] M. Bonferroni[2]

[1]*Azienda Ospedaliera S.Croce e Carle, CUNEO, Italy;* [2]*Hematology, CUNEO, Italy*

Heparin-induced thrombocytopenia (HIT) is an acquired hypercoagulable state. As soon as HIT is suspected, heparin should be discontinued and non-heparin anticoagulant should be started. Lepirudin, Argatroban and Danaparoid are among the alternative anticoagulants more investigated in HIT. Dermatan sulphate (DS) is a safe, effective and inexpensive therapeutic option for HIT though clinical experience with its use is limited. We report our clinical experience on DS therapy in patients with HIT. HIT with thrombotic syndrome was clinically suspected in 3 patients according with the Warkentin's criteria (NEJM 2001;344:1286-1292). Laboratory confirmation of HIT was obtained by an ELISA test for anti-PF4-heparin antibodies in two patients. Venous thromboembolism was confirmed by imaging tests. DS(Mediolanum Farmaceutici, Milan, Italy) was administered by intravenous continous infusion(initial dose 0,6 mg/Kg/h), the infusion was regulated monitoring APTT every 6-12 hours and targeting APTT of 1,5-2 times the normal value. Platelet count was close monitored. Warfarin was started in two cases when platelet count rised over 100 x 10^9 /L, in one case before DS infusion. DS was stopped when INR was in therapeutic range for two consecutive values. The cost by vial of DS is 0.9175 euro in Italy Patients included were two females (59 and 81 years old) and one male (65 years old). In one case HIT developed during administration of unfractionated heparin for lower limbs deep venous thrombosis (DVT) and the course was complicated by sinus thrombosis, in two cases after antithrombotic prophylaxis with low-molecular-weight heparin: one, after abdominal neoplastic surgery suffered of lower limbs DVT and pulmonary embolism, the other, with entero-vesical fistula, suffered of upper limbs deep vein catheter-related thrombosis and pulmonary embolism. In 2 patients DS was started with platelet count of 49-129 x 109 /L and platelet count rised over 150 x 109 /L after 3-5 days, DS infusion was continued for 11 and 13 days; in patient ,taking Warfarin, DS was started with platelet count 44 x 109 /L and after 2 days was stopped with platelet count of 62 x 109 /L. No bleeding complications or adverse events were observed, clinical improvement was observed in all patients. Patient with entero-vesical fistula, few weeks later HIT, was operated and antithrombotic prophylaxis with DS offered an uneventful outcome. DS total therapy cost for two patients, before warfarin was in therapeutic range, was respectively of 26.7 euro and 56 euro. DS appears an effective and safe therapeutic option for patients with HIT. Our experience, together with other reports in which patients with HIT were treated successfully, encourages using this alternative anticoagulant drug in HIT therapy and as postoperative antithrombotic prophylaxis in patients with recent history of HIT. DS shows also a favourable profile cost-benefit.

1239

THROMBOMODULIN, SEPCR AND DI-DIMERS AS PLASMA MARKERS OF ENDOTHELIAL DYSFUNCTION IN WOMEN WITH A HISTORY OF RECURRENT PREGNANCY LOSS

K. Sfiridaki, M. Houstoulaki, H. Kteniadaki,
K. Livadiotaki, E.V. Stavrakaki, E. Kandidaki

Venizelion Hospital, HERAKLION, Greece; Hemostasis and Thrombosi Department, Blood Bank Center, Venizelion Hospital, Heraklion Crete, Greece

Recurrent pregnancy loss can be associated with endothelial disturbance (whether activation, dysfunction or damage). Thrombomodulin (TM) and the endothelial protein C receptor (EPCR) are glycoprotein receptors expressed mainly on the endothelial surface of blood vessels and also in the placenta. They both play a key physiological role in the protein C anticoagulant pathway. Defects in these proteins might play an important role in the pathogenesis of fatal loss. So we decided to study Di-Dimers, TM and SEPCR as early markers for the beginning and prognosis of recurrent pregnancy loss. We studied 102 women with unexplained fetal loss and 44 women as control group. We used an immunologic assay (Dade Behring) for calculating Di-Dimers and ELISA (Asserochrom ASTAGO) for TM and SEPCR measurement. The levels of Di-Di were 189,28±13,7 μg/L in patients group and 172,02 μg/L in control group (p=0,46). TM levels were 9,78±2,4ng/mL in patients group and 8,3±2,7ng/ml in controls (p=0,017). There was not statistical difference in SEPCR measurement (187,2 ng/ml versus 160,7 ng/mL in control p=0,22). TM may serve as a clinically meaningful endothelial injury marker in women with a history of recurrent pregnancy loss. Further investigation is needed to see the significance of other factor as Di-Di and SEPCR.

1240

THE ABO BLOOD GROUPS, FVIII, FIX, VWF LEVELS AND LEIDEN IN PATIENTS WITH THROMBOSIS IN GREECE.

K. Sfiridaki, M. Houstoulaki, H. Kteniadaki,
K. Livadiotaki, E.V. Stavrakaki, E. Kandidaki

Venizelion Hospital, HERAKLION, Greece

Increased levels of FVIII, FIX, vWF in plasma as the presence of FV leiden represent an important risk factor for venous thromboembolic disease. There is also a relationship between these factors and ABO Blood groups. We investigated the influence of the ABO blood group in Greek patients with thrombosis and the association with raised plasma levels of the above coagulation factors. 159 patients of median age 43,5 + 5,4 y (97 females and 62 males) were included in our study. They have visited our hospital for first thrombotic event when younger than 60 years old. Patients with malignancies or with history of liver failure or nephrotic syndrome were excluded. As controls 60 (36 F and 24M) apparently healthy individuals were recruited. We measured the plasma levels of

FVIII, FIX, vWAg and also we determined the ABO type blood and the presence of FV Leiden. There is a high prevalence of thrombosi, in the non-O blood group (102/57), especially in patients of A or AB type blood. The plasma levels of FVIII, IX and vWF were 112,6%, 82,4%, and 136,5% respectively in the patients group and 92,5%, 89,2% and 99,5% in control group. There were 14 carriers of FV Leiden (8,7%) in patients group and 3 carriers in healthy controls (3,3%). We conclude that : 1.there is association between non-O blood type and thrombosis in Greek patients; 2. FVIII, vWF levels and FV Leiden were significant higher in individuals with non-O blood type.

1241

THE CONTRIBUTION OF PROTHROMBIN AND MTHFR MUTATIONS TO VENOUS AND ARTERIAL THROMBOEMBOLISM IN CARRIERS OF FACTOR V LEIDEN

A. Taher, Z. Otrock, R. Mahfouz, I. Khalil, W. Shamseddeen, Z. Kanaan

American University of Beirut Medical Ct, BEIRUT, Lebanon

Backgrounds. Given the multifactorial aspect of thrombophilia, the identification of combined genetic factors in patients with thrombotic episodes is important to a more accurate risk assessment. These patients are routinely screened for Factor V Leiden (G1691A), prothrombin G20210A, and MTHFR C677T gene mutations at the American University of Beirut Medical Center (AUBMC). It is not known, however, if the presence of prothrombin or MTHFR gene mutations increases the risk in carriers of Factor V Leiden mutation whether they had venous or arterial thrombosis. *Aims.* We assessed the contribution of prothrombin and MTHFR to the thrombotic risk in patients with venous or arterial thromboembolism. *Methods.* The population in our study consisted of a group of patients presenting with thrombosis over a period of 18 months. Patients were screened for the three most common thrombophilia genetic mutations namely Factor V Leiden (G1691A), prothrombin G20210A and MTHFR C677T. A group of healthy controls was also included in the analysis. The DNA of patients and controls was extracted using the PEL-FREEZ extraction kit (PEL-FREEZ, DYNAL, USA) and stored at -80°C for later use. Simultaneous testing for all three mutations was done using the Reverse Hybridization StripAssay (Vienna Lab). Extraction, PCR amplification, and Hybridization steps were all followed upon the recommendation of the manufacturer.

Table 1. Clinical presentation of patients with thrombosis.

Arterial thrombosis	*Number of patients*
Cerebrovascular accident	25
Peripheral vascular disease	9
Myocardial infarction	7

Arterial thrombosis	*Number of patients*
Deep vein thrombosis	47
Superficail thrombophlebitis	13
Pulmonary embolism	12
Portal vein thrombosis	8
Sagittal sinus thrombosis	5
Transverse sinus thrombosis	4
Subclavian vein thrombosis	4
Central retinal thrombosis	3
Mesenteric vein thrombosis	3

Results. The sample included 41 patients with arterial thrombosis, 99 patients with venous thrombosis, and 125 healthy controls. The average age of the three groups was 42.8→19.4, 39.6→17.5, and 35.4 →18.6 years respectively. Table 1 shows the clinical presentation of patients with thrombosis. Patients with venous thrombosis were 7.1 times more likely to have Factor V mutation (heterozygous or homozygous) as compared to the control group. On the other hand, neither prothrombin nor MTHFR mutation was significantly associated with venous thrombosis. A logistic regression was conducted to test whether prothrombin and MTHFR mutations increase the risk of venous thrombosis among subjects with Factor V mutation. None of the two factors was found to be significantly associated with venous thrombosis after controlling for Factor V. Patients with arterial thrombosis were 2.5 and 4.4 times more likely to have Factor V (p=0.04) and prothrombin (p=0.04) mutations as compared to the control group. There was no significant association between MTHFR mutation and arterial thrombosis. Upon controlling for Factor V in the logistic regression, prothrombin mutation was 5.3 times more likely to be present among patients with arterial thrombosis. Conclusions. In Lebanon, the presence of prothrombin and/or MTHFR mutations does not seem to influence the risk of venous thrombosis in Factor V Leiden carriers. However, in patients with arterial thrombosis, the risk is increased in Factor V Leiden carriers with prothrombin mutation. These results might have an influence on the risk assessment and management of patients with arterial thrombosis. However, no final conclusions can be made from our results because of the small sample size. It would also be rational to conduct similar studies with stratification of patients into subgroups based on the definite site of thrombosis.

1242

ARE SEMINAL FACTORS IX AND IXA INVOLVED IN THE SEMINAL COAGULUM FORMATION?

A. Lwaleed,[1] B. Lwaleed,[1] A. Goyal,[2] G. Delves,[2] R. Greenfield,[3] A. Cooper[2]

[1]*Southampton University Hospital NHSTrust, SOUTHAMPTON, United Kingdom;* [2]*University of Portsmouth, PORTSMOUTH, United Kingdom;* [3]*American Diagnostica Inc, STAMFORD, CT, USA*

Backgrounds. In spite of evidence demonstrating the importance of the seminal coagulation and liquefaction process in terms of global fertility and that the seminal coagulum is composed of fibrin-like material, it has rarely been studied from the conventional haemostatic factors perspective. *Aim:* To investigate Factor (F) FIX and FIXa in human semen. *Materials and Methods.* Using a one stage factor assay based on PT/APTT and spectrozyme fIXa assay FIX and FIXa were studied in a total of 119 semen specimens obtained from sub-fertile, normally fertile, fertile sperm donors and vasectomy subjects. *Results.* Both FIX and FIXa were quantifiable in human semen. There was a wide individual variation in FIX and FIXa levels within groups. Despite the group size, statistically significant associations with fertility-related parameters were infrequent. There is a positive correlation between FIX and its activation product, FIXa (n=36; r=0.51; p<0.05). Factor IXa elevation in the high sperm-clump group was significant (p<0.05) and days of abstention correlated with FIXa levels (n=63; r=0.3; p<0.05). *Conclusion*: The key finding of this study is that both FIX and FIXa are present in concentrations not dissimilar to plasma levels and apparently functional, as the activated form is also present.

1243

PREVALENCE OF FACTOR V G1691A, FACTOR II G20120A AND MTHFR C677T POLYMORPHISMS IN PATIENTS WITH DEEP VENOUS THROMBOSIS

A. Agorasti,[1] I. Bazdiara,[2] D. Pantelidou,[2] G. Trypsianis,[2] D. Margaritis,[2] E. Spanoudakis,[2] A. Anastasiadis,[2] A. Goutzouvelidis,[2] D. Konstantinidou,[1] C. Tsatalas,[2] G. Bourikas[2]

[1]*General Hospital of Xanthi, XANTHI, Greece;* [2]*Democritus University of Thrace, ALEXANDROUPOLIS, Greece*

Backgrounds. The risk of thrombosis may occur through the interaction of both genetic and acquired factors. The predisposition towards thrombosis increases with the number of risk factors present in the patient. *Aim*: The aim of this study was to evaluate the independent and combined effect of factor V G1691A (FV-Leiden, FVL), factor II G20210A (PTH) and methylene-tetrahydrofolate reductase C677T (MTHFR) polymorphisms on the incidence of deep venous thrombosis (DVT). *Patients and Methods.* We enrolled 128 patients with first episode of DVT (65 males, 63 females) and 186 healthy individuals (83 males, 103 females). FV, FII and MTHFR genotypes were analyzed using PCR amplification. We calculated odds ratio (OR) with 95% confidence intervals (CI), adjusted for gender and age by means of multiple logistic regression. *Results.* The prevalence of the heterozygote and homozygous variants for FVL (25,0% vs 6,5%, p< 0,001) and PTH (10,2% vs 3,2%. p=0,011) were higher among DVT patients compared with controls. However, the presence of the T/T genotype for MTHFR was not different between the two groups (9,4% in patients vs 8,1% in control group, p=0,684). In order to evaluate independent and combined effect of the above mutations on the incidence of DVT, we divided the entire cohort into seven groups according to the presence of none, one or two mutations. The combination of the three mutations was not detected. The group without any mutation was used as reference group. Both FVL and PTH significantly increased the risk for DVT compared to the reference group (FVL: OR=4.0, 95%

CI=1.9-8.4, $p<0.001$; PTH: OR=3.8, 95% CI=1.3-10.6, $p=0.012$), while MTHFR was not significantly associated with DVT (OR=1.1, 95%CI=0.4-2.7, $p=0.831$). Moreover, the combination of FVL with PTH and FVL with MTHFR further increased the risk of DVT compared to the reference group (FVL and PTH: OR=14.5, 95% CI=1.7-29.9, $p=0.013$; FVL and MTHFR: OR=10.3, 95% CI=1.2-20.1, $p=0.034$), as well as compared to FVL or PTH only. *Conclusions.* Our results indicated that FVL and PTH, but not MTHFR, were important independent risk factors for DVT. In addition, the combination of FVL with PTH or MTHFR further increased the odds of development of DVT.

1244

GELATINOUS BONE MARROW TRANSFORMATION IN A PATIENT WITH LEUKOPENIA AND ANOREXIA: CASE REPORT AND REVIEW OF THE LITERATURE

I. Teyssandier,[1] M. Hummel,[2] C. Kuhn,[2] C. Lorentz,[2] L. Di Renzo[3]

[1]*Labo. hmatologie, Hpital Htel-Dieu, PARIS, France;* [2]*III. Medizinische Universitätsklinik, MANNHEIM, Germany;* [3]*Dipartimento di Medicina Sperimentale, ROMA, Italy*

Backgrounds. Gelatinous bone marrow transformation (GMT) is a rare disorder of unknown pathogenesis, characterized by fat cell atrophy, focal loss of hematopoietic cells, and deposition of extracellular gelatinous substances Histochemically the amorphous background consists of mucopolysaccharides, rich in hyaluronic acid. The spectrum of underlying diseases are heterogeneous and age-dependent but GMT is commonly associated with weight loss and cachexia. It is still unclear whether the gelatinous transformation is a primary or secondary change but it may be a reversible lesion if the underlying disorder can be eliminated and an adequate nutritional intake be reestablished. *Patient/case report*: We are presenting a case of gelatinous transformation of the bone marrow in a 40-year-old female who presented with cachexia, night sweats and leukopenia. The receptionist of a gynaecological outpatient clinic and mother of three children had a history of 5 kg weight loss within the last 2 years. Furthermore she reported about amenorrhea since 5 years, hypothyroidism and lack of zinc. Alcoholism or nicotine abuse was denied. On physical examination she was 170 cm tall and weight was 47 kg (body mass index [BMI],16 kg/m^2). Her skin was dry and physical examination was remarkable for an aphthous ulcer of the bottom lip and acid defects of the adamantine caused by excessive consumption of lemon juice. The thyroid was without pathological findings. Laboratory data showed leukopenia (white blood cells, 2.6/nl, hemoglobin 14 g/dl, hematocrit 38,4%, platelets 160/nl, neutrophils 52%, lymphocytes 42%, monocytes 4%, eosinophils 2%). Liver function tests were abnormal: Serum aspartate aminotransferase (AST) 53 U/l (<31U/l), alanine aminotransferase (ALT) 56 U/l (<34 U/l), lactate dehydrogenase (LDH) 321U/l (<248 U/l), Vitamin B12 (1282 mg/l; normal 200-1100) and folate (16.5 mg/l; normal 2,5-17) were elevated. Zinc was decreased (9.7 mmol/l, normal 11-23 mmol/l). TSH and free thyroxine were normal under substitution. Auto-antibody tests were not elevated. Viral studies, including HIV, hepatitis, CMV and EBV, were negative. An abdominal ultrasound examination was normal. A bone marrow aspirate stained with May-Grünwald Giemsa showed decreased cellularity and abundant amorphous, eosinophilic material (Figure 1).

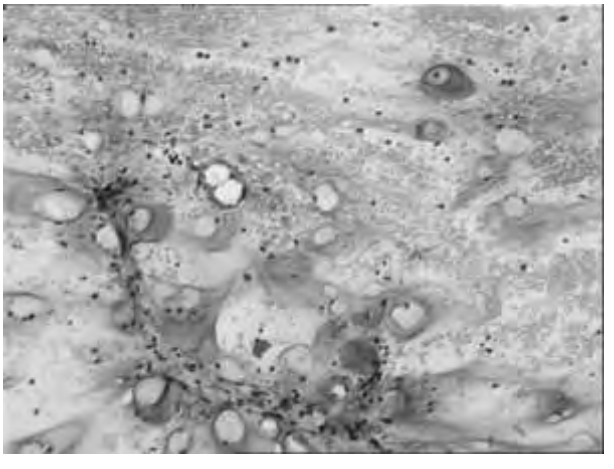


Figure 1. Bone marrow aspirate stained with May-Grünwald Giemsa.

The amorphous gelatinous substance was identified as acid mucopolysaccharide on alcian blue staining at pH 2,5. On a bone marrow trephine biopsy granulopoiesis was reduced and in the intratrabecular space eosinophilic extracellular material and fat cell atrophy, consistent with gelatinous bone marrow transformation was found. *Summary/Conclusions.* GMT is a rare disorder that is associated with various underlying diseases, the most frequent being anorexia nervosa and the acquired immunodeficiency syndrome (AIDS). Although frequently associated with weight loss, the bone marrow changes have not been associated with any specific deficiency state and their pathogenesis has not been fully elucidated. GMT may act as an indicator of severe illness in a patient but is not indicative of a particular disease. It is an uncommon cause of cytopenia and should be considered in the setting of malnutrition.

1245

WHAT ARE THE DECISION MAKING PROCESSES OF HAEMATOLOGY PATIENTS WHEN ASKED TO PARTICIPATE IN A PHASE III CANCER CLINICAL TRIAL, IN A DISTRICT GENERAL HOSPITAL

N. Singer

NHS Lanarkshire, Scotland, GLASGOW, United Kingdom

The objective of this research was to explore the decision making processes of patients who have been asked to consider participating in a Phase III Haematology cancer clinical trial. This was done, by undertaking a qualitative study, using semi-structured interviews in a District General Hospital, within the West of Scotland. Seven participants were purposively selected to participate who had been newly diagnosed with a Haematological malignancy. An extensive literature review was undertaken prior to the study with the decision made to conduct semi-structured interviews. These were conducted with participants and tape-recorded. Participants were asked about their decision making processes when they were approached to consider taking part in a Phase III Haematology cancer clinical trial. A thematic analysis was conducted out using the long-table approach. The following themes emerged from the study: The timing of the request to participate, the effect of altruism, the process of randomisation and the quality of information that is provided to potential participants. All had an impact on the decision making process of patients when considering participating in a Phase III trial. The findings of this study suggest that further research into why patients choose not to participate in Phase III trials is worthy of consideration. Furthermore the implementation of a training intervention programme aimed at improving healthcare professionals communication with cancer patients is also recommended.

1246

HEPATITIS B VIRUS REACTIVATION AFTER CHEMOTHERAPY AND IMMUNOTHERAPY IN NON-HODGKINS LYMPHOMA: REPORT OF TWO CASES

M. Vakili, A.H. Faghihi Kashani, A. Zargar Koucheh

Iran University of Medical Sciences, TEHRAN, Iran

Backgrounds. Reactivation of hepatitis B virus infection in subjects receiving cytotoxic treatment for hematological malignancies occurs in 20'50% of chronic HBsAg carriers and in an unknown number of HBsAg negative subjects harbouring occult HBV infection. Immunotherapy with monoclonal antibodies against CD epitopes on lymphocytes produces deep immunosuppression. *Case presentation*: This paper reports two patients suffering from Non-Hodgkin's Lymphoma (NHL). Patient no.1 was a 62 y/o man, a case of NHL and chronic HBsAg carrier, too. He received four courses of chemotherapy with fludarabine without serious complication but because of poor response and CD20 positivity in more than 80% of lymphocytes, rituximab, an anti-CD20 monoclonal antibody prescribed for him. After the 4th course of therapy, severe hepatitis developed. Viral study revealed increased serum HBV-DNA from 12×10^3 to $>10\times10^6$ copies/mL. Before starting lamivudine, patient died due to hepatic failure and encephalopathy. Patient no.2 was a 47 y/o man with a questionable history of HBsAg positivity about 25 years ago but this test was negative prior to chemotherapy. He received CHOP regimen and rituximab and after the 6th course of therapy severe hepatitis developed. Viral study revealed positive HBsAg and HBV-DNA $>100\times10^6$ copies/mL. Lamivudine 100mg/day started but after one week he died because of massive uncontrollable widespread bleeding. Conclusion: Considering results of the published data and a high rate of hepatitis B virus reactivation in cancer patients undergoing chemotherapy and immunotherapy, it is necessary to evaluate hepatitis B and C viral markers including at least HBsAg, HBsAb and HBcAb and HCV Ab pri-

or to therapy and also an international protocol for managing patients at risk for reactivation of hepatitis B virus in high prevalent areas such as Iran should be carried out.

1247

A 3 YEAR SURVEY OF STRAINS IDENTIFIED IN BLOOD CULTURES IN A CLINICAL HEMATOLOGY UNIT

R. Jeddi, L. Thabet, H. Benneji, A. Turki, R. Belakhal, L. Aissaoui, H. Benabid, Z. Belhadjali, B. Meddeb

Aziza Othmana Hospital, TUNIS, Tunisia

Febrile neutropenic cancer patients are at the risk for development of serious infections, morbidity and mortality. Among these infections; bacteraemia had a place of choice and is associated with a strong mortality. The microbiological documentation is not always present, the antibiotherapy remain probabilist inspired of the ecology of the service. The aim of this study is to analyze the bacteriological profile of bacteraemia in a clinical haematology unit in order to guide better the antibiotherapy of first intention. All the microorganisms(n=138) collected over 3 years(January 2003 to December 2005), from blood cultures of hospitalized patients in the clinical haematology unit were studied. Antimicrobial susceptibility testing has been carried out by disk diffusion method as referred to the French Society of Microbiology. All susceptibility data were stored in a laboratory data base using Whonet software. Duplicate isolates defined as the same bacterial species for the same patient with the same profile of susceptibility were excluded. Gram positive cocci rate(GPC) was 60,1% and Gram negative bacilli(GNB) 39,9%.Evolution in time showed an equal rate between GPC and GNB in 2003(51,4% versus 48,6%), then an increase of GPC rate isolated in bacteraemia was observed in 2004 (63,6%) and 2005(63%).The most frequently identified species were coagulase-negative staphylococci(CNS): 42,7%, *Pseudomonas aeruginosa*:10,9%, *Klebsiella pneumoniae*:10,9%, *Escherichia coli*:8,7% and *Staphylococcus aureus*:8,7%. The rate of methicillin resistant staphylococci was 25% in S.aureus and 50, 8% in CNS; no VISA (vancomycin intermediate *S. aureus*) was detected during the study period. *P. aeruginosa* resistance was 33, 4%, 30,8%, 40% respectively for ceftazidime, imipeneme and amikacin. Concerning K.pneumoniae, 86,7% of strains were resistant to ceftazidime, 46,7% to ciprofloxacin and 85,7% to amikacin. The frequencies of resistance to ceftazidime, ciprofloxacin and amikacin of *E.coli* were respectively 50%, 33,3% and 41,7%.Imipeneme and colistin were the most active agents against *K. pneumoniae* and *E. coli* (resistance rate= 0%). Bacteremia were mainly caused by coagulase-negative staphylococci during the three years study. Multiresistance of germs isolated is worrying limiting the therapeutic choice. Ongoing cooperation between haemotologists and microbiologists is important to detect trends in epidemiology wich can be used to design empirical antibiotic regimens and guide infection control policies.

1248

BACTERIAL FLORA AND ANTIBIOTIC RESISTENCE IN A CLINICAL HEMATOLOGY UNIT

R. Jeddi, L. Thabet, H. Benneji, A. Turki, L. Aissaoui, R. Belakhal, H. Benabid, Z. Belhadjali, B. Meddeb

Aziza Othmana Hospital, TUNIS, Tunisia

Infections are among the most serious complications in neutropenic patients and are associated with an increased morbidity and mortality. Ongoing surveillance of infection in neutropenic patients is essential to detect changes in epidemiology and to guide better empirical antibiotic regimens and infection control policies. The aim of this study is to analyze the bacterial flora and the antibiotic resistance of isolates in a clinical haematology unit during three years period. From 1 January 2003 to 31 December 2005, 437 strains were isolated from different specimens. Antimicrobial susceptibility testing has been carried out by disk diffusion method as referred to the French Society of Microbiology. All susceptibility data were stored in a laboratory data base using Whonet software. Duplicate isolates defined as the same bacterial species for the same patient with the same profile of susceptibility were excluded. Gram negative bacilli(GNB) rate was 47,1% and Gram positive cocci (GPC) rate 52,9%.The most frequently identified species were Coagulase negative staphylococci(CNS): 29,3%, *Esherichia coli*:14%, *Staphylococcus aureus*: 10,7%, *Klebsiella pneumoniae*: 9,1% and Pseudomonas aeruginosa:7,5%. The global rate of methicillin resistant Staphylococci was 27,7% in *S. aureus* and 61,4% in CNS, no VISA (vancomycin intermediate *S. aureus*) was detected during the study period. For *E. coli*, the frequencies of resistance to ceftazidime, ciprofloxacin and amikacin were respectively: 45%, 26,3% and 21,3%.Concerning *K. pneumoniae*, 84, 8% of strains were resistant to ceftazidime and were producing extended spectrum β-lactamase (*BLSE*). The evolution in time showed an increase in rate of *K. pneumoniae BLSE*: 57, 1% in 2003 versus 95, 5% in 2005.All strains of *K. pneumoniae* isolated remained sensitive to imipenem and colistin. Concerning *P. aeruginosa*, 50% of strains were resistant to ceftazidime, 50% to imipenem, 51, 6% to ciprofloxacin and 54, 5% to amikacin. An increase of the imipenem resistance in *P. aeruginosa* was observed from 2003 to 2005(28, 6% in 2003 versus 45, 5% in 2005). The incidence of antimicrobial resistance has markably increased during 2005, especially for the ceftazidime in *K. pneumoniae* (95, 5% in 2005 versus 57, 1% in 2003) and the imipenem in *P. aeruginosa* (45, 5% in 2005 versus 28, 6% in 2003). After this study, a restriction of the use of ceftazidime wich utilized in the first antibiotherapy was instaured in the unit. The ongoing surveillance of antimicrobial resistance in the hematology unit should be helpful in formulation of effective guidelines for therapy.

1249

PROPHYLAXIS OF INVASIVE FUNGAL INFECTIONS (IFI) IN ACUTE NON LYMPHOID LEUKEMIA (ANLL): EFFICACY OF AMPHOTERICINE B LIPID COMPLEX (L-AMB) SINGLE LARGE DOSE DURING INDUCTION

A. Chierichini, S. Fenu, V. Bongarzoni, M. Cedrone, M. Bartolini, M.P. Persiani, B.R. Ronci, B. Anaclerico, S. Cortese, L. Annino

Azienda Ospedaliera S.Giovanni Addolorata, ROME, Italy

Up to now ,the optimal prophylactic regimen to prevent IFI in ANLL is not yet been identified. The L-AmB has been used in patients refractory or intolerant to other antifungal drugs, although long time is required to resolve overt infection and this event influence ANLL therapeutic plan. The efficacy of L-AmB seems to be related both to improved tissue penetration and to sustained bioactivity of drug levels in lung ,brain, kidney, liver, spleen (*Anaissie et al. 2004*). On the basis of this issue ,we have planned a pilot study for IFI prophylaxis in *de novo* ANLL to test the efficacy and safety of a single large dose of L-AmB (15 mg/kg) during post induction neutropenia. Primary endpoint: to evaluate the incidence of fungal infection according to International Consensus (*Ascioglu et al. 2002*) during and up to four weeks after prophylaxis. *Patients*: the study started in may 2004 and it is still open ;as of January 2006, 21 consecutive adult ANLL (4 APL) patients - 14 M,7 F, median age 57 yrs (range 39-75) - are enrolled. Intensive induction chemotherapy included standard/high dose cytosine-arabinoside + antracyclines + etoposide and retinoic acid + antracyclines in APL. *Methods.* Inclusion criteria were: 1) neutropenia (PMN < 0.5×10^9/L) longer than ten days; 2)surveillance cultures, mannano and galattomannano antigens negative; 3) no fever and/or clinical signs of infection. On the day after the end of induction, patients received a single dose of L-AmB (Ambisome, Gilead™) at 15 mg/kg i.v. A second dose was repeated 15 days later in those cases persistently neutropenic and who met inclusion criteria. L-Amb PK profile was tested in 15 patients at the following times: 0,1,4,24 hours, 7th and 14th day from drug administration. *Results.*15/21 patients(72%) achieved complete haematological remission,1 partial remission, 1 was resistant and 4 died during induction aplasia .Overall median duration of neutropenia was 22 days(range 16-42).The median dosage of L -AmB was 900 mg /dose (range 750-1200 mg); in six cases a second single dose was administered. Of 21 patient entered in this study,17 (81%) did not developed fungal infection , 3 cases had IFI (1 Candida spp sepsis and 2 Aspergillosis, respectively) and 1 died early. As L-AmB related toxicities, only 2 patients had CTC grade II allergy, promptly recovered by i.v. steroids. The median L-AmB PK results are, to date, available in 8 patients: 0 h <0,15, 1 h 8,92±4,25; 4h, 51,26 ±26,7; 24h, 3,92±11,77; 7th, d 1,39±1,97; 14th, d, 0,27±0,092; (*lower standard rate 0,15 mg/l ± standard deviation*). *Comments*: The reported results, if obtained in a single center, seems to show that L-AmB single large dose is an effective and safe approach in IFI prophylaxis. In fact 81% of treated patients did not develop fungal infections and side effects were not significant. Furthermore the preliminary results of PK profile demonstrated an early high plasma levels of the drug which slowly cleared until 14th day.

1250

RECURRENT DISSEMINATED SKIN LESIONS DUE TO METARRHIZIUM ANISOPLIAE IN AN ADULT PATIENT WITH ACUTE MYELOGENOUS LEUKEMIA

S.O. Osorio,[1] R.C. de la Camara,[2] C. Granados,[1] M. Cuenca,[3] M.C. Monteserin,[1] N. Somolinos,[1] L. Benito,[1] J.A. Garcia-Vela,[1] L. Garcia-Alonso,[1] M.D. Menor,[1] R. Serrano,[1] C. Gonzalez-Herrada,[1] C. Garcia,[1] F. Oña[1]

[1]*Hospital de Getafe, GETAFE: Madrid, Spain;* [2]*Hospital de la Princesa, Madrid, Spain;* [3]*National Institute Carlos III, MAJADAHONDA, Madrid, Spain*

Case report. A 62 year old male was diagnosed in April 2005 with acute myelogenous leukemia. On day +42 after two cycles of induction chemotherapy, disseminated skin papules with central ulceration appeared involving the face, trunk, and limbs. A skin biopsy yielded dermoepidermic necrolysis and fibrin thrombi. Not cultures were obtained. The patient was receiving antibiotics and caspofungin for persistent neutropenic fever. He became afebrile and recovered from his neutropenia four days later. Caspofungin and antibiotics were withheld. The skin lesions gradually improved. Two more cycles of chemotherapy were administered and two new lesions appeared after the fourth cycle. They gradually resolved. During September and October 2005, while awaiting for an autologous stem cell transplantation (SCT), disseminated skin lesions reappeared. A new skin biopsy was performed and was initially interpreted as an acute inflammatory dermal lesion with a mixed neutrophilic and histiocytic infiltrate. During this time most lesions underwent spontaneous resolution, but new papules appeared. On November 2005 he was admitted to undergo an autologous SCT. He had then 4 skin lesions in resolution. Deeper sections of the second skin biopsy revealed a nidus of fungi in the dermis with broad hyphae. A new skin biopsy showed similar features . Biopsy cultures and a galactomannan test were negative. A chest CT scan was normal. Tissue samples were sent to the Mycology Reference Laboratory of Spanish National Center for Microbiology. Specimens were analysed using a panfungal PCR-based assay designed to amplify the internal transcribed spacer regions 1 and 2 from fungal rRNA gene complex. Subsequent sequencing of amplified fragments and comparison with sequences of other fungal species included in databases led to know that the DNA amplified from tissues belonged to the fungal species *Metarrhizium anisopliae*. During transplantation no new lesions appeared. On day +9 he was started on liposomal amphotericin B for neutropenic fever but it was discontinued after one dose because of a severe infusion reaction. He did not receive any further antifungal therapy and the skin lesions resolved. In December 2005, he showed 2 new skin papules. A biopsy was performed and cultures and PCR for fungal DNA were negative. Voriconazole was started and the lesions disappeared. Treatment was discontinued after a month. No further skin lesions appeared. *Discussion*: We report the first case of a probable disseminated infection caused by M. anisopliae in an adult patient. The organism was not isolated from cultures and was identified by PCR techniques. This case exemplifies the clinical usefulness of molecular methods to diagnose mycosis due to emerging pathogens. Metarrhizium anisopliae is a common insect pathogen and occasionally causes infection in animals and humans. To date, there are only 5 reported cases of disease in humans: two of keratitis, two of sinusitis, and one of a disseminated invasive infection in an immunocompromised child. There is no standard treatment. Susceptibility testing suggests that *M. anisopliae* may be resistant to amphotericin B, 5-Fluocitosine, and fluconazole. Itraconazole and Voriconazole could be more effective agents.

1251

AUDIT OF THE USE OF ANTIFUNGALS AND THE ACTUAL RATES OF FUNGAL INFECTION ACCORDING TO EORTC/MSG CRITERIA IN PATIENTS UNDERGOING HIGH DOSE CHEMOTHERAPY FOR AML AND/OR ALLOGENEIC TRANSPLANTATION

N. Farah,[1] D. Tsitsikas,[1] M. Heath,[1] A. Stiles,[1] S. Agrawal[2]

[1]*Barts and the London NHS Trust, LONDON, United Kingdom;* [2]*St. Bartholomew's Hospital, LONDON, United Kingdom*

Background. The high morbidity and mortality of fungal infections in neutropenic patients has led to prophylactic and empirical drug regimens. Antifungal drug use and actual rates of invasive fungal infection (IFI) may differ considerably. *Aims*. To audit the use of antifungal drugs in patients with AML and/or allograft transplant recipients admitted to our hospital from 01/01 to 31/12/2004 and to apply the EORTC/MSG criteria (1) for IFI. *Methods*. The medical notes were retrospectively reviewed for: conversion from prophylactic to empirical treatment; time to neutrophil recovery; duration of hospitalisation and mortality. Primary prophylaxis was with fluconazole 400mg od, while empirical therapy (ambisome) was prescribed for persistent neutropenic fever despite 72-96 hours of antimicrobials. Those patients, who did not meet EORTC/MSG *possible* criteria, were termed *unlikely*. Galactomannan testing was not done routinely in our hospital. *Results*. 54 patients (out of 77 eligible) were assessable, providing 137 episodes: 75% underwent intensive chemotherapy for AML/MDS, 18% allogeneic transplantation and 7% supportive treatment for neutropenic sepsis on the background of AML. 114/137 episodes (83%) received primary prophylaxis - oral fluconazole (78%); 21/137 (15)% secondary prophylaxis - oral voriconazole; 2% did not receive prophylaxis. The conversion rate from prophylaxis to empirical therapy was 25% (35/137), mainly due to persistent neutropenic fever. These 35 episodes concerned 27 of the 54 patients (50%). The EORTC/MSG infection rates are shown in Table 1.

Table 1.

EORTC-defined infection	*Number of episodes that were started on empirical treatment*
Proven	0/35 (0%)
Probable	4/35 (11%)
Possible	18/35 (51%)

Of these 35 episodes only 4 (11%) were *probable* IFI - all had HRCT evidence of IFI with negative bacterial cultures. Another 22 episodes involved HRCTs: 11 had normal results, and 11 had non-specific changes. Thus, HRCT was the main diagnostic test in the small total number of *probable* infections. Patients in 25 of the 35 episodes on treatment (71%) were hospitalised for >28 days (range 29-60, median 34). Their EORTC/MSG IFI score was: 13 *possible*, 3 *probable*, 6 *unlikely* and 3 not documented. In 3/35 episodes (9%) patients on treatment died during admission - none were related to IFI (2 died of AML; 1 of non-fungal pneumonia); all had *possible* IFI. Time to neutrophil recovery (> 0.5 x 10^9/L) in 20/35 episodes on treatment (57%) was ≥ 22 days (range 22-41, median 28): 10 had *possible* IFI, 2 *probable*, 6 *unlikely* and 2 were not documented. *Summary/Conclusions*. The EORTC/MSG IFI rate - possible/probable/proven - was only 22/137 (16%) episodes. This may be due to effective prophylaxis and/or early initiation of empirical treatment, but it also reflects the fact that the EORTC/MSG criteria were not intended for routine clinical use. Our audit data aims to allow regular review of anti-fungal policies, but is limited by its retrospective nature. Consequently, we have introduced prospective, continuous audit and will present our preliminary findings of the introduction of voriconazole as primary prophylaxis. Furthermore, we will outline an ongoing study combining galactomannan, PCR and measurement of inflammatory markers in blood, broncho-alveolar lavage and exhaled breath condensate for the early diagnosis of invasive aspergillosis.

References

1. Ascioglu S et al. Clin Infect Dis 2002;34:7.

1252

FUNGAL INFECTIONS DIAGNOSTIC IN A NECROPSY STUDY. COMPARISON WITH THEIR CLINICAL SUSPICION

M.J. Moreno,[1] F.A. Arriba,[1] V.P. Pérez,[1] A.J. Jerez,[1] M.L. Lopez[1], C.V. Vallejo,[1] F.P. Pastor,[2] J.M. Moraleda,[1] V. Vicente[1]

[1]*Hospital Morales Meseguer, MURCIA, Spain;* [2]*Hospital Reina Sofa, MURCIA, Spain*

Backgrounds. Intensive therapies on haematology illnesses treatment conditioned an increase on fungal infection incidence and, occasionally, an aggressive medical profile. Samples that allows to establish the microbiologic or/and anatomopathologic diagnosis are not always easy to collect, and frequently an empiric treatment has to be started, based only on a suspect diagnosis. *Aims.* To compare the correlation between the suspicion of invasive fungal infection (IFI) and its clinical manifestations with the findings of the autopsy, in patient with malignant haematology diseases. *Patients and Methods.* We study 34 demised patients, 24 diagnosed patients of Acute Leukemia and 10 with other hematologic neoplasias that had been summited to an autologous progenitor cell

transplantation. In 16 of the 34 patients the fungal infection was suspected at the beginning. According to the EORTC diagnostic criteria for IFI, 12 patients (75%), had a possible IFI and 4 cases (25%) presented a probable IFI. There were no cases with a proven IFI before death. *Results.* The autopsy demonstrated the presence of fungal infection in 10 patients: in 7 cases there was a clinical suspicion of fungal infection while in three cases it was an unexpected discovery in the autopsy. The organs shown up by the autopsy to be affected by the fungal infection were: lung (9 cases), digestive (6 cases), heart (2 cases), kidney (2 cases), CNS (2 cases) liver (2 cases) spleen (1 case), mediastinic mass (1 case), and pancreas (1 case). It is relevant that in most patients, the organic involvement other that lung was not suspected before their death, and it was responsible for very outstanding clinical manifestations during the end stage of the illness: superior vena cava syndrome (1 case), serious heart arrythmias (1 case), profuse diarrhea (1 case), renal failure (1 case), and hepatic failure (1 case). *Conclusion*: Our study shows high incidence of clinical suspected IFI at the end-stage disease not confirmed with the autopsy, and the complexity of the clinical manifestations associated to this type of infections.

1253

CD40 LIGAND AND CALCIUM IONOPHORE TREATMENT OF DENDRITIC CELLS FROM HEALTHY DONORS AND PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE AND MULTIPLE MYELOMA

L. Kovarova, J. Michalek, M. Penka, R. Hajek

University Hospital, BRNO, Czech Republic

Backgrounds. Dendritic cells (DC) are the most potent antigen-presenting cells that can initiate adaptive immune response. They can differentiate from peripheral blood precursors and as an immature dendritic cells react to wide range of stimuli. Upon the activation/maturation process they change their phenotypic, morfologic and functional characteristics. The ability to acquire and activate blood DCs makes them a valuable source for future immunotherapy trials, but there are inconsistent reports about the functional state of dendritic cells from patiens with multiple myeloma (MM). *Aims.* Comparison of 48h treatment of immature dendritic cells with different stimuli as CD40 ligand (CD40L) and calcium ionophore (CI). Searching for differences in phenotype of DCs from healthy donors and patiens with MGUS and MM after stimulation. *Methods.* Ficoll-Hypaque-separated peripheral blood mononuclear cells (PBMC) from 10 healthy donors and 12 patients (7 MM and 5 MGUS) were used. Adherent precursors of DCs were cultured with GM-CSF and IL-4. CD40L and/or CI were added in day 1 or 4 to generate mature DCs. Multicolor flowcytometric analysis was done in day 0 and after harvest of DCs in day 3 or 6. Following monoclonal antibodies were used: CD11c, CD80, CD83, CD86, lineage mixture, CCR2, CCR5, CCR7, IL-12, MIP-1a, HLA-DR. *Results.* The highest percentage of CD83, characteristic marker of mature DCs, was found in 3rd day of culture after stimulation CD40L and also CI. In the 6th day was the average percentage of CD83 decreased to the half of 3rd day. There was found no differences between donors and patients. Expression of HLA-DR was relatively constant, independent on the time of the harvest or type of the stimulation and again without differences between groups of patients and donors. Expression of costimulation molecule CD80 slowly increased in 6th day of culture after CD40L stimulation, but CD86 was higher after CI stimulation. Chemokines receptors CCR2 and CCR5, markers of immature DCs, were expressed in low density as well as CCR7, marker of mature DCs. There was some evidence, that CCR7 was increased in healthy donors. Production of cytokine IL-12 and chemokine MIP-1a were also low. *Summary/conclusion*. Addition of CD40L and/or CI to an immature DCs obviously didn´t evoke their maturation, because there were found no strong expression of CCR7, IL-12 and MIP-1a. We didn´t found significant diferences between DCs generated from healthy volunteers and patients.

Funding: Supported by grant MZCR NR/8081-3.

1254

THE PREPARATION OF MYELOMA-SPECIFIC CYTOTOXIC T CELLS BASED ON INTERFERON γ PRODUCTION

D. Ocadlikova,[1] L. Zahradova,[1] L. Kovarova,[1] M. Penka,[2] R. Hajek,[1] J. Michalek[3]

[1]*LEHABI, BRNO, Czech Republic;* [2]*Department of Clinical Hematology, BRNO, Czech Republic;* [3]*Cancer Immunobiology Center, DALLAS, USA*

Backgrounds. Autologous hematopoietic stem cell transplantation has been considered recently as part of a standard treatment strategy in patients with multiple myeloma (MM). Here we attempted to enhance the immunotherapeutic potential of autologous T cells based on selection of myeloma-reactive lymphocytes *in vitro*. *Aims.* The aim of this study was to identify and characterize autologous myeloma-reactive T cells *in vitro* and to evaluate their cytotoxic effect. *Methods.* Irradiated myeloma cell line ARH 77 or patient's myeloma cells were used as tumor antigen for dendritic cells loading. Peripheral blood mononuclear cells of 8 healthy volunteers and 10 MM patients were used for repeated stimulation of T lymphocytes. Activated T cells producing interferon γ were isolated using immunomagnetic separation (MACS) (Miltenyi Biotech) and expanded *in vitro* by phytohemaglutinin and high concentrations of interleukin 2. A specific cytotoxicity against myeloma cells was tested after the expansion with propidium iodide or 7-amino actinomycin D. Activated T cells were labeled with CFSE. Allogeneic T cells and interferon γ negative fraction of T cells served as controls. *Results.* In an allogeneic setting with ARH 77 cells the enrichment of interferon γ positive T cells by magnetic beads in healthy donors started from a median of 2.83% (1.97-4.58%) to 48.57% (15.14- 82.98%) after MACS and from 1.91% (1.14-3.4%) to 73.14% (3.9-88.75%) after MACS in $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells, respectively. Interferon γ positive T cells were further expanded *in vitro* from 0.5×10^6 to a median of 160×10^6 (150×10^6-420×10^6) T cells within 4 weeks and the test of cytotoxicity has demonstrated a high degree of specific killing of ARH 77 myeloma cells 69.17% (38.04-78.23%). Cytotoxicity of expanded interferon γ negative T cells was negligible. In an autologous setting with autologous myeloma cells used as an antigen, the enrichment of interferon γ positive T cells from MM patients started from 1.12% (0.27-6.2%) to 7.85% (0.42-12.6%) after MACS and from 1.9% (0.37-14.4%) to 14.7% (1.28-71.4%) after MACS in $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells, respectively. Interferon γ positive T cells were expanded in vitro from 0.12×10^6 (0.05×10^6-0.4×10^6) to 88.5×10^6 (35×10^6-226×10^6) within 8-12 weeks and the test of cytotoxicity has demonstrated only a modest specific killing of autologous multiple myeloma cells (18.88%) and allogeneic ARH 77 cells (18,21%). *Conclusions.* These data demonstrate a promising tumor-specific effect of allogeneic myeloma-reactive T cells but only a modest effect in an autologous setting in patients with MM. Whether that is due to a low MACS enrichment or low immunogenicity of autologous myeloma cell needs to be further clarified.

Funding: Supported by the grant: IGA MZCR 1A/8907-5.

1255

AMINO ACID SEQUENCES OF T CELL RECEPTOR REACTING AGAINST MULTIPLE MYELOMA

S. Dudova,[1] L.K. Kovarova,[2] R.H. Horak,[2] R.H. Horvath,[3] M.P. Penka,[4] R.H. Hajek,[5] D. Ostroveanu[6]

[1]*FN Brno, BRNO, Czech Republic;* [2]*LEHABI, FN Brno, BRNO, Czech Republic;* [3]*Genex CZ, BRNO, Czech Republic;* [4]*OKH, FN Brno, BRNO, Czech Republic;* [5]*IHOK, FN Brno, BRNO, Czech Republic;;* [6]*Fundeni Clinical Institute, BUCHAREST, Romania*

Backgrounds. Multiple myeloma (MM) is a disease caused by malignant proliferation of B lymphocytes in the bone marrow. Recently, high-dose chemotherapy with autologous hematopoietic transplantation has been considered a standard treatment for patients with advanced stages of MM. Such treatment delays relapse but it is not curative and almost all patients ultimately develop recurrent disease. Based on preclinical and clinical studies it is evident that myeloma-reactive T lymphocytes play an important role in immunologic response to this malignant disease. Myeloma-reactive T lymphocytes have been shown to be a promising approach in adoptive cellular immunotherapy aside autologous transplantation of bone marrow graft. *Aims.* Our aim was to analyse T cell receptor (TCR) sequences reacting against multiple myeloma. Experimental study was performed in 10 patients to provide information on the specificity and spectrum of recognized antigens. *Methods.* Dendritic cells loaded with apoptotic bodies from magnetically isolated myeloma cells have been used to stimulate autologous T lymphocytes. Activated myeloma-specific T cells were identified and expanded. After mRNA isolation the anchored reverse transcription using modified version of SMART method was done. PCR product was cloned into plasmid vector, transformed in bacterial cells and individual clonotypes were sequenced. *Results.* Oligoclonality of TCR receptor was demonstrated in myeloma specific *in vitro* expanded T lymphocytes, in one case monoclonal population of tumor specific T cells was found. These findings support the assumption of myeloma specific antigens stimulating only certain autologous T lymphocytes. *Conclusions.* Structural characterization of TCR receptor of myeloma specific clones provides further evidence for the role of these T lymphocytes in immunotherapy. Receptor

sequence determination can be used as a marker for evaluation of the vaccine strategy.

Funding: This work was supported by IGA MZCR 1A/8709-5.

1256

CRITERIA FOR CORD BLOOD DONOR SELECTION ON THE BASIS OF ROC CURVE ANALYSIS

M. Solves,[1] A. Perales,[2] V. Mirabet,[1] M. Arnao,[3] A. Soler,[1] R. Roig[1]

[1]*Transfusion Centre, VALENCIA, Spain;* [2]*Hospital Universitario la Fe, VALENCIA, Spain;* [3]*Hospital de la Ribera, ALZIRA, Spain*

The main limitation factor for a wide use of umbilical cord blood (CB) for transplantation is the cell dose. In this sense, many cord blood banks have set a total nucleated cells (TNC) content ranging from 60 to 100 x 107 as minimum required values for storing the units. In order to optimise cord blood banking and reduce the number of UCB units deferred before processing, an effort in donor selection is mandatory. Many authors have showed that placental and neonatal weight influence hematopoietic content of cord blood units. To establish obstetric criteria for selection of cord blood units before cryopreservation. In order to determine the optimal placental and neonatal weight for selecting cord blood donors according to the number of TNC, we have performed Receiver Operating Characteristic (ROC) curve analysis. ROC curve is a graphical technique commonly used to find optimal cut off value of a test using sensitivity and specificity data. We thought it could be useful to determine cut off values of placental and neonatal weight for an optimal selection of UCB units.

Table 1.

TNC×10⁷	*Cut-off*	*Area under the*	*95% confiance*
60×10⁷			
Neonatal weight	≥ 3190	0.635±0.013	0.616-0.653
Placental weight	≥ 646	0.685±0.013	0.666-0.704
70×10⁷			
Neonatal weight	≥ 3195	0.638±0.012	0.619-0.656
Placental weight	≥ 645	0.682±0.012	0.662-0.701
80×10⁷			
Neonatal weight	≥ 3195	0.632±0.011	0.614-0.651
Placental weight	≥ 635	0.676±0.011	0.656-0.695
90×10⁷			
Neonatal weight	≥ 3195	0.631±0.011	0.612-0.649
Placental weight	≥ 635	0.648±0.011	0.629-0.668
100×10⁷			
Neonatal weight	≥ 3195	0.624±0.011	0.605-0.642
Placental weight	≥ 635	0.637±0.011	0.617-0.657

Results. We revised 2590 cord blood units collected at Valencia Cord Blood bank for a four-year period. Mean TNC content of UCB before processing was 107.65→54.74×07. Mean neonatal weight and placental weight were 3313.36→430.7 g. and 652.2→122.1 g. ROC curve analysis was performed with MedCalc software for windows v. 7.4.2.0. Variable was considered 0 or 1 if TNC was < or > 60, 70, 80, 90, and 100×107, respectively and classification variables were considered placental weight and neonatal weight. Results are shown on the following Table. We conclude this statistical analysis can be helpful to determine cut off value of placental/neonatal weight according to the required limit of TNC for each bank. This approach would reduce the number of collected units that are refused before processing.

1257

ANALYSIS OF THE CD34+ CELLS CONTENT OF THE CORD BLOOD UNITS STORED IN A REGIONAL CORD BLOOD BANK

M. Solves,[1] V. Mirabet,[1] M. Arnao,[2] F. Carbonell-Uberos[1], D. Planelles,[1] A. Soler,[1] R. Roig[1]

[1]*Transfusion Centre, VALENCIA, Spain;* [2]*Hospital De La Ribera, ALZIRA, Spain*

Some clinical studies have shown that graft selection should be based principally on $CD34^+$ cell dose and grafts should contain at least 1.7 x 10^5 $CD34^+$ cells per kilogram of recipient´s body weight. However, criteria for selecting collections suitable for freezing and storage are not standardized. Although most banks have set a total nucleated cells (TNC) content ranging from 60 to $100x10^7$ as initial minimum required values for storing the units, only a few banks selecting cord blood units on the basis of their $CD34^+$ cell content. Aims. To analyse the $CD34^+$ cell content of the cord blood units stored at the Valencia cord blood bank and the characteristics of the units according to their $CD34^+$ cell content. We reviewed the data of 2149 cord blood units stored at Valencia cord blood bank and selected on the basis of their TNC content (more or equal than $100×10^7$). $CD34^+$ cells were quantified by flow cytometry. CB sample was taken directly from the bag and after volume reduction-before cryopreservation and 5×10× cells were incubated using monoclonal antibodies conjugated CD45 fluorescein and CD34 phycoerythrin (Becton Dickinson) and 7 amino-actomicin D as marker of DNA staining. Flow cytometric analysis was performed using Cell Quest software. ProCount progenitor cell enumeration kit was used in comparison with our standard protocol, giving similar results. Total CD34+ cells content was calculated by multiplying the CD34 percentage per TNC. A total of 2149 cord blood units were stored for a 5 years-period. Mean TNC, $CD34^+$ cell percentages and total $CD34^+$ cells were$112.37→37.17×10^7$, $0.36 → 0.25\%$ and $41.79→34.74×10^5$, respectively. From these units, 489 (22%) had a total $CD34^+$ cell content less than $20×10^5$. Characteristics of the units according to their CD34 cell content are shown in the table. Conclusions. In order to increase the quality of cord blood units stored, the CD34 cell content should be considered as a selection criteria of cord blood units for cryopreservation and storing.

Table 1.

CD34+ Content x 10e5	N (% of stored units)	CD34+ x 10e6	CD34+ (%)
< 20	489 (22.5%)	12.18 ± 5.49	0.13 ± 0.06
≥ 20	1660 (77.6%)	50.51 ± 34.92	0.43 ± 0.24
≥ 30	1218 (56.6%)	59.80 ± 36.53	0.49 ± 0.25
≥ 40	865 (40.2%)	69.98 ± 38.96	0.55 ± 0.27
≥ 50	610 (28.4%)	80.53 ±4 2.1	0.62 ± 0.29
≥ 60	434 (20.2%)	91.15 ± 45.8	0.68 ±0.32
≥ 70	292 (13.6%)	104.14 ± 50.99	0.74 ± 0.36
≥ 80	223 (10.3%)	113.14 ± 55.33	0.79 ± 0.39
≥ 90	160 (7.4 %)	124.40 ± 61.81	0.84 ± 0.44
≥ 100	106 (4.9%)	139.60 ± 71.34	0.88 ± 0.52

1258

MESENCHYMAL STEM CELLS CONTRIBUTE TO THE HEALING PROCESS AND FUNCTIONAL IMPROVEMENT OF ISCHEMIC INJURED KIDNEY IN RAT MODEL

S.K. Park, S.B. Bae, C.K. Kim, N.S. Lee, K.T. Lee, J.H. Won, D.S. Hong, H.S. Park, J.K. Kim

Soonchunhyang University Hospital, BUCHEON-SI, GEYONGGI-DO, South-Korea

Objective. Renal failure is a common disease with high morbidity and mortality. Ischemic injury is one of the most common cause of renal failure. Recent studies have reported that adult bone marrow-derived cells can contribute to renal remodeling and a dramatic repopulation of the mesangium. Moreover, there was a report that the role of bone marrow-derived hematopoietic stem cells in the regeneration of the renal tubular epithelium after ischemic injury in mice. When ischemic injury is inflicted on targeted organ, MSCs may migrate to the site of damage, undergo differentiation, and promote structural and functional repair. We evaluated whether bone marrow-derived MSCs contribute to the healing process and improve renal function in injured kidney of rat by ischemia. *Materials and Methods.* Right nephrectomy was performed in six-week-old SD rat. And the left renal artery and vein were clamped for 45 min followed by 2/3 nephrectomy was done and then clamp releases to allow perfusion. MSCs prelabeled with green fluorescent protein (GFP) injected via tail vein. Peripheral blood was collected serially for evaluation of blood urea nitrogen and creatinine and functional evaluation was done with radioisotope renal scan. Histologic study and confocal microscopic evaluation were performed at 4 days, 1 week, and 4 weeks after MSCs injection. *Results.* We demonstrated that GFP positive cells were detected in damaged kidney by confocal microscopy and engrafted MSCs promoted healing process by ischemic injury. Also engrafted MSCs differentiated into tubular epithelial cells, thereby restoring renal structure. In the group with MSCs injection, the levels of blood urea nitrogen and creatinine were lower than control group without MSCs injection (BUN Day 4, control group; 65.0±8.1, MSC infusion group; 31.1±5.1). And MSCs injected rats demonstrated that renal func-

tion recovered more rapid and more close to the normal value in radioisotope renal scan. *Conclusions.* The results presented here suggest that MSCs are capable of healing and functional restoring of damaged kidney by ischemic injury. So MSCs may be useful for cell therapy of renal failure.

1259

IMMUNOREACTIVITY TO ANTI-FIBRONECTIN AND ANTI-LAMININ POLYCLONAL ANTIBODIES IN PARAFFIN EMBEDDED MICE BONE MARROW ARE DEPENDENT ON HISTOLOGICAL PROCESSING

M.E. Favero,[1] R.A. Fock,[2] M.A.R. Vinolo,[2] M.L.Z. Dagli,[2] P. Borelli[2]

[1]*Universidade Estadual de Londrina, LONDRINA, Brazil;* [2]*Universidade de So Paulo, SO PAULO, Brazilz*

Immunohistochemistry (IH) is an useful tool to study tissues and organs and it has been widely used in researchs or to supplement classical morphologic diagnosis including pathological conditions of the hemopoietic system. However, applicability of IH in bone marrow analyses presents some technical limitations, because some antigens are *masked* during tissue processing (fixation, decalcification and paraffin embedding) making the applicability of this methodology unfeasible. Bone marrow microenvironment contains cells of different tissues (bone, hemopoietic tissue and stromal elements) and several extracellular matrix (ECM) substances, mainly glycoproteins, proteoglycans and cytokines. The composition of bone marrow ECM is topographically variable and is associated to the development of different lineages of blood cells, suggesting the existence of specific interactions between ECM, stem cells and stromal elements. In previous studies, we have shown that bone marrow from mice submitted to protein malnutrition underwent structural changes with decrease in cellularity and increase of glycoproteins extracted from ECM. The aim of this work was to evaluate the influence of fixative and time of fixation in the antigenic preservation of extracellular matrix glycoproteins fibronectin and laminin, and their distribution in bone marrow of mice. Esternum of well nourished Swiss mice, 2 to 3 months old, were fixed with 3 different fixatives: Methacarn (1 hour), 10% neutral buffered formalin pH 7,2 (1 hour, 6 hour or 24 hours) and 4% buffered paraformaldeyde pH 7,2 (24 hours). Decalcified using 5% nitric acid (3 hours) or 10% buffered EDTA, pH 7,2 (7 days) and then processed routinely with standard dehydration and embedding in paraffin. Tissue sections (5 micron thick) mounted on silane coated slides were dewaxed, rehydrated, and brought to phospathe buffered saline. Endogenous peroxidase activity was blocked by incubation for 30 minutes in 3% hidrogen peroxide. Sections were incubated with primary antibodies against fibronectin (1:400) and laminin (1:25) overnight at 0-4°C. After washes in PBS, slides were incubated with biotinylated secondary antibody for 30 minutes, washed in PBS and incubated with a streptavidin-biotin complex coupled to peroxidase for 30 min. Peroxidase activity was revealed with diaminobenzidine. Slides were counterstained with Harris hematoxylin. No immunoreactivity, for both anti-laminin and anti-fibronectin antibodies, was detected in any specimen fixed in 4% paraformaldeyde (24 hs) and decalcified with EDTA. Sections fixed in Methacarn showed strong background reaction for laminin immunostaing and a false nuclear pattern for fibronectin immunostaining. Among the used conditions, adequate morphological and antigenic preservation of fibronectin and laminin were achieved on sections fixed in 10% buffered formalin during one hour and decalcified in 5% nitric acid. Tissue processing stages can significantly influence on immunoreactivity of antibodies against fibronectin and laminin. This way, sections fixed in 10% buffered formalin during one hour and decalcified in 5% nitric acid were selected to compare bone marrow ECM glycoproteins distribution *in situ* of nourished and malnourished mice.

Funding: Financial support: FAPESP, CAPES.

1260

VORICONAZOLE (VCZ) PROBABLY DOES NOT AFFECT THE PHARMACOKINETICS OF METHOTREXATE (MTX)

Y. Jabali,[1] N. Mallatova,[1] V. Smrcka,[1] A. Vrzalova[2]

[1]*Regional Hospital, CESKE BUDEJOVICE, Czech Republic;* [2]*University Hospital Motol, PRAGUE, Czech Republic*

Background and aim: Both MTX & VCZ have many drug interactions. Whether an interaction exists between them is not known. The aim of the study was to explore whether oral VCZ affects the pharmacokinetics of MTX. *Patients and Methods.* With informed parental consent, a prospective study with standard clinical & drug monitoring was performed on 2 children with intermediate-risk, B-cell-precursor acute lymphoblastic leukemia during consolidation chemotherapy. This consisted of 6-mercaptopurine 25 mg/m^2/d PO 56 d, MTX 2 g/m^2/24h IV q 2 wk x4, leucovorin 15 mg/m^2 x3 IV per each MTX & MTX 12 mg IT q 2 wk x4. The first child (case) has been on oral VCZ because of proven invasive pulmonary aspergillosis (IPA). The other (control) was infection free. MTX serum levels [µmol/L] were measured by fluorescence polarization immunoassay at 0, 18, 24, 36 & 42h of starting the infusion until achieving a concentration of ≤0.25. Inter- & intra-patient MTX levels were compared by Wilcoxon signed ranks test & Friedman test, respectively. *Results.* A 10.5-yr-old boy developed IPA, controlled by ABLC (29 d). Oral VCZ for 61 d was given thereafter (150 mg bid first d, then 100 mg bid 60 d). Consolidation started while being 17 d into VCZ treatment. Another 5-yr-old boy w/o IPA was undergoing identical consolidation under the same conditions. 3 pairs of MTX infusion running in parallel were evaluated. Baseline MTX levels were always below the detection limit (<0.05) in both. In the VCZ/MTX arm, MTX levels at 18, 24, 36 & 42h were, respectively: 28.62, 19.35, 0.52, 0.18 (1. MTX); 27.62, 24.17, 0.79, 0.25 (2. MTX); 23.13, 21.38, 0.46, 0.16 (3. MTX). The 4. MTX was delivered 5 d off VCZ, yielding a concentration of 21.53, 11.39, 0.32 & 0.11 at those time points, respectively. In the MTX-only arm, the corresponding figures were 24.14, 19.50, 0.62, 0.22 (2. MTX); 27.09, 18.63, 0.50, 0.23 (3. MTX); 26.60, 18.77, 0.50, 0.19 (4. MTX). All MTX levels were in the expected range, with non-significant (NS) inter-patient difference (p=0.7; 0.07; 0.5). However, while the intra-patient difference was not significant (p=0.1) in the control, it was so (p=0.02) in the case because of significant (p=0.02-0.04) depression in levels of the last MTX (off-VCZ) vs the first 3 ones (on-VCZ), within which the intra-patient difference was NS either (p=0.1). Within 1-3 d of every MTX infusion, the first pt developed cheilitis and photosensitivity over exposed body parts, the severity of which was related to intensity of sunshine. This reaction always resolved towards the next MTX. No other side effects were observed, nor IPA did exacerbate under VCZ/MTX. Assessment of VCZ levels is planned. *Conclusions.* Although the off-VCZ levels of MTX differed significantly from those on-VCZ in the case, this could be attributed to the well known intra-patient inter-dose variability in MTX disposition. The bulk of data suggests that oral VCZ seems not to affect MTX pharmacokinetics significantly. However, this should be confirmed on a larger number of pts and/or doses of MTX given during VCZ therapy.

1261

CELL DIFFERENTIATION AND APOPTOSIS OF U-937 LEUKEMIA CELL LINES BY A NEW COMPOUND FROM DENDROSTELLERA LESSERTII

M. Mahdavi, R. Yazdanparast

Institute of Biochemistry and Biophysics, TEHRAN, Iran

Recently, we have reported on the activity of 3-hydrogenkwadaphnine (3-HK), purified from Dendrostellera lessertii, to induce differentiation and apoptosis in HL-60 cells upon a single dose treatment at a drug concentration of 2.5-40 nM. Regarding the relatively weaker potency of 3-HK compared to that of the crude extract, we looked for additional compound(s) with similar properties in the crude extract. Herein, we report on the isolation of a second and a more potent compound, with differentiation capability and apoptotic effects. The new compound inhibited growth and proliferation of U937 cells with an IC50 of 1.75 µg/ml. The new compound, at 0.5-2.5 µg/mL inhibited proliferation of U937 cells by more than 70% and their viabilities were decreased by 47±2.1% after 72 h of treatments. The new compound also induced differentiation of U937 to monocyte/macrophage-type cells as became evident through phorbol ester-dependent reduction of NBT, morphological changes as examined by Wright-Giemsa staining and expression of CD11b and CD14 as analyzed by flow cytometry. The results indicated that treatment of U937 cells with the new compound for 3 to 4 days induced apoptosis as assayed qualitatively by acridine orang/ ethidium bromide (Ao/EtBr) double staining, agarose gel electrophoresis and quantitatively by Annexin V technique using flow cytometry. Based on these observations, *D. lessertii* could be a novel candidate for pharmaceutical evaluation.

1262

A TRIAL TO IDENTIFY SIDE EFFECT OF DRUGS AT ULTRA-EARLY STAGE

M. Matsumoto

Matsumoto Living Cell Research Laborator, TOKYO, Japan

Method. Using the M-H Method, living blood cells are taken from a patient, and divided into two layers, upper and lower (i.e., ULRBC(U)

and LLRBC(L): hereinafter referred to as *U* and *L*). Then, each layer of blood cells is put into 3 mL of RPMI-1640 solution and cultured in a 37°C 5%-CO2 incubator. Assuming that the entire amount of daily dosage of drug administered to the patient (α) is absorbed in 5 L of blood, the absorbed amount of drug in 3 ml of blood (X) is calculated as follows: X:3=αmg:5000, i.e. X=3×α/5000. The calculated amount of drug (X) is added to 3 ml of physiological saline, and fully mingled together. The solution, which the drug is dissolved, is sterilized by filtering in a clean bench. The sterilized solution is put into U and L, and cultured in a 37°C 5%-CO2 incubator. They are diachronically monitored with an inverted phase-contrast microscope, and recorded in photos and VCRs. U and L which the drug-dissolved solution is not added to are used as the control groups. *Results.* Compared with the control groups, if the drug is harmful for the red blood cells, RBC shows deformation and degeneration earlier, and dies after getting into the cells like *firefly*, *ghost* and *black shell*; i.e., the life of RBC is shortened. The WBCs (white blood cells) grow to enormous size in various complicated shapes, staying alive for three to five weeks. Moving Micro Livings' and *Mysterious chains* emerge in some cases. Compared with the control groups, *Photo-Cytosis Phenomenon (named by Matsumoto)* occurs sooner in some cases and later in other cases. *Conclusions.* If the proposed method is clinically adopted, individual variation against side effect of drugs can be determined early. It would be secure and risk-free to check all of the drugs administered for four or more weeks using the proposed method before administration. I believe medical science in the 21st century will go on in this direction. If the proposed method is started to use at the point of drug development, it can cut down on waste and expenses drastically.

1263

UNEXPECTED SUBACUTE LEUCOENCEPHALOPATHY FOLLOWING INTRATHECAL METHOTREXATE AND CYTARABINE ADMINISTRATION IN A PATIENT HOMOZYGOUS FOR MTHFR 677C→T POLYMORPHISM

M. Bonifacio,[1] A. Andreoli,[1] S. Friso,[2] P. Guarini,[2] A. Simonati,[3] M. Ruggeri,[4] G. Nadali,[1] G. Pizzolo[1]

[1]*Section of Hematology, VERONA, Italy;* [2]*Section of Internal Medicine, VERONA, Italy;* [3]*Dpt of Neurological and Visual Science, VERONA, Italy;* [4]*Section of Psychiatry and Clin Psychol, VERONA, Italy*

Background. Intrathecal administration (ITA) of chemotherapeutics, mainly Methotrexate (MTX) and Cytarabine (Ara-C), is a standard approach to central nervous system (CNS) prophylaxis of aggressive Non Hodgkin Lymphomas. MTX is known to cause diffuse symmetrical leucoencephalopathy in children. A genetic variant of Methylenetetrahydrofolate reductase (MTHFR) due to the 677C→T polymorphism determines a striking reduction in the enzyme activity and has been associated with increased toxicity during MTX administration in children. Reports of neurotoxic effects in adults are lacking, despite the increasing use of aggressive protocols in this age group. Aim. To report the case of an adult, carrying the MTHFR homozygous mutant 677TT genotype, who developed subacute leucoencephalopathy following intrathecal prophylaxis with MTX and Ara-C. Case report. A 32-year-old Caucasian male was diagnosed with aggressive B-cell lymphoma (stage IV B, bone marrow, liver and spleen involvement). Treatment was planned according to modified POG 8617 regimen (Todeschini G, Ann Oncol, 1997). Course A included CNS prophylaxis with intrathecal MTX (12 mg) and Ara-C (50 mg) at days 1 and 4. CSF biochemical examination was normal and cytospin was acellular. Six days after day 4 ITA, the patient became acutely confused and showed behavioural and speech disturbances. Motor impairment of the right leg was also recorded. Psychiatric assessment excluded a psychiatric origin for this disorder. Body temperature was normal and he had no signs of meningeal involvement. No signs of infection were found. Brain CT and MRI were unremarkable. EEG showed a pattern of diffuse cerebral dysfunction. Speech and behaviour disturbances improved starting from day +16, without complete recovery. Day +60 brain SPECT showed diffuse hypoperfusion, most evident at parieto-temporal regions bilaterally. At day +71 the patient had a seizure. EEG showed predominantly frontal disturbances. Brain MRI, performed 109 days after day 4 IT, showed bilateral hyperintense lesions in subcortical white matter in T2-weighted images. The molecular analysis detected a MTHFR 677TT homozygous mutant genotype. *Discussion*. The present case report indicates the possible event of severe CNS damage in adults undergoing ITA administration of MTX. The mechanisms underlying MTX toxicity remain uncertain. Review of possible mechanisms behind methotrexate neurotoxicity points towards several metabolic pathways affected by the drug. MTHFR is crucial to folate metabolism, essential for DNA synthesis and repair pathways as well as DNA methylation and its severe deficiency results in hyperhomocysteinemia, which can cause neurotoxicity. Although we cannot completely rule out the possible role of cytarabine in causing CNS toxicity in our patient, the clinical and radiological findings suggest the major role of methotrexate. Ara-C neurotoxicity is mainly reported in children and there is a preferential involvement of the spinal cord rather than the brain. The presence of the TT homozygosity in our case seems to confirm the predisposing role of this genotype for CNS damage in adults, extending the observations made in children. Given the high prevalence of the homozygous 677TT genotype in the Italian population, ITA MTX prophylaxis should be carefully followed-up and leucovorin rescue in adults should be considered.

1264

FIP1L1-PDGFRA POSITIVE CHRONIC EOSINOPHILIC LEUKEMIA IN TUNISIAN PATIENTS

S. Menif

Institut Pasteur, TUNIS, Tunisia

Hypereosinophilic syndromes (HES) are a heterogenous group of rare disorders characterized by sustained and otherwise unexplained overproduction of eosinophils with organ involvement and consecutive dysfunction. Detection of the FIP1L1-PDGFRA fusion gene or the corresponding cryptic 4q12 deletion in HES supports the diagnosis of chronic eosinophilic leukemia CEL and provides a molecular explanation for the pathogenesis of this disorder. We screened 11 tunisian patients fulfilling the WHO criteria of HES for the presence of the *FIP1L1-PDGFRA* fusion gene using nested reverse transcription polymerase chain reaction on peripheral blood samples. 4 of the 11 patients (36%) were positive for this fusion gene .Sequence analysis revealed a substantial heterogeneity of the fusion transcripts due to the involvement of several FIP1L1 exons .all patients were male.the median age at diagnosis was 34 years (range,18-50);one patient had a history of hypereosinophilia of more than 11 years .2 patients had clinically important and symptomatic eosinophilic endomyocardial disease with thrombotic events. Splenomegaly was constant in *FIP1L1-PDGFRA* positive CEL but not in the other HES patients (only 1/7). Recent reports document the efficacy of low doses of imatinib mesylate (100mg/day) in FIP1L1-PDGFRA+ CEL patients .All patients who present with sustained non reactive hypereosinophilia should be screened for the FIP1L1-PDGFRA fusion gene.

1265

TWO NEW MUTATIONS IN THE DIA1 GENE IN PATIENTS WITH RECESSIVE CONGENITAL METHEMOGLOBINEMIA TYPE I AND II

C. Vercellati,[1] E. Fermo,[1] P. Bianchi,[1] A.P. Marcello,[2] O. Marangoni,[3] M Garatti,[1] A Zanella[1]

[1]*Fondazione IRCCS Ospedale Maggiore, MILAN, Italy;* [2]*Fonfazione IRCCS Ospedale Maggiore, MILAN, Italy;* [3]*Presidio Ospedaliero S. Giovanni Bianco, BERGAMO, Italy*

Recessive congenital methemoglobinemia (RCM) is caused by deficiency of reduced nicotinamide adenine dinucleotide (NADH)-cytochrome b5 reductase (cytb5r, EC 1.6.2.2) The cytb5r gene (DIA1) is localised on chromosome 22q13-qter and contains nine exons; tissue-specific alternative transcripts originate 275-amino acid soluble and 300-amino acid membrane-bound isoforms. Cytb5r deficiency exists in two distinct clinical forms: in Type-I the enzyme deficiency is restricted to the red cell-soluble cytb5r isoform and results in cyanosis; in Type-II the enzyme defect involves both the soluble and membrane-bound cytb5r isoforms, and severe mental retardation and neurological impairment are also present. More than 35 different mutations of DIA1 gene have been reported in RCM: disruptive mutations (stop, frameshift, splicing) are associated with the severe Type-II disease.The aim of this study is to describe two cases of methemoglobinemia type I and type II respectively, associated with two new mutations of the DIA1 gene. The cytb5r activity in the red cells was measured using the NADH-ferricyanide method. Molecular analysis of DIA1 gene was performed on blood DNA by PCR and direct sequencing. *Case 1*: the propositus was a male infant of Northern Italian origin born at 38 weeks from spontaneous delivery. Cyanosis was noted at birth accompanied by reduced oxygen saturation (stable on 90-95%), but normal pO2 on arterial blood gas analysis and no evidence of cardiopulmonary abnormality. Weight at birth was 2710g and physical examination revealed neither developmental delay nor microcephaly. Methemoglobinemia was detected (26.3%). Haemoglobin electrophoresis failed to demonstrate an M band. Studies on red blood cells revealed marked NADH-cytb5r deficiency (0.9 IU/gHb; normal range 15.36-23.23.06 IU/gHb) while the parents exhibited levels of

9.3 and 10.8 IU/gHb. The infant started on daily oral methylene blu (3 mg/kg/die) and methemoglobin level remained < 7%. Molecular analysis of DIA1 gene showed the presence of the new homozygous missense mutation GGC-GAC at codon 143, resulting in the aminoacidic substitution Gly143Asp. The mutation was present in parents at the heterozygous level. Consanguinity was denied but it can be supposed since the parents originated from the same little enclave. *Case 2*: a male, was born at term as second child of consanguineous Egyptian parents (I grade cousins). At the age of 5 months, the baby presented the clinical symptoms of RCM type II: cyanosis associated with profound mental retardation, microcephaly, bilateral athetosis, strabism, frequent vomiting and crying. Studies on RBC revealed marked NADH-cytb5r deficiency (1.4 IU/gHb) while his mother, father and healthy sister exhibited levels of 10.1, 5.8 and 10.7 IU/gHb respectively. Molecular analysis of DIA1 gene showed the new homozygous intronic mutation IVS5+2 t-c which probably results in splicing alterations and absence of functional protein, and may therefore account for the severe clinical pattern.

1266

CELL CYCLE STATE OF HEMATOPOIETIC PROGENITOR CELLS AND BONE MARROW LYMPHOCYTE PHENOTYPE IN PATIENTS WITH ACQUIRED APLASTIC ANEMIA

A.D. Kulagin,[1] N.V. Pronkina,[1] I.V. Kruchkova,[1] S.A. Sizikova[1], V.V. Denisova,[1] A.V. Gilevich,[1] V.S. Kozhevnikov,[1] I.A. Lisukov,[2] V.A. Kozlov[1]

[1]*Institute of Clinical Immunology, NOVOSIBIRSK, Russian Federation;* [2]*State Medical University, NOVOSIBIRSK, Russian Federation*

Backgrounds. Acquired aplastic anemia (AA) is associated with profound quantitative and functional defects in the hematopoietic stem cell compartment due to T-cell-mediated suppression. *Aim:* The aim of the study was to evaluate cell cycle state of hemopoietic progenitor cells and phenotype of bone marrow lymphocytes in patients with AA during immunosuppressive treatment (IST) and after allogeneic bone marrow transplantation (alloBMT). *Methods.* The study was performed in 33 patients (19 male, 14 female) including 9 with non-severe AA (NSAA), 19 with severe AA (SAA) and 5 with very severe AA (VSAA). There were 5 cases of hepatitis-associated AA and 2 cases of pregnancy-associated AA. In 26 cases, no cause of AA was identified. The median age at diagnosis was 20 years (range, 13-53 years). Combined IST with repeated courses of horse antithymocyte globulin (ATG) and cyclosporine A (CsA) was used for 19 patients with SAA and VSAA. Nine patients with NSAA were treated with CsA alone. AlloBMT from related matched sibling was performed in 4 young patients with SAA/VSAA. The phenotype of BM lymphocytes and the number of CD34-positive and HAE-9-positive erythroid cells were determined by single or double color flow cytometry analysis. Cell cycle and apoptosis of CD34+ and HAE-9+ cell populations were studied by using the 7-amino actinomycin D (7AAD). As controls 14 healthy age-matched volunteers were studied. *Results.* The overall survival is 94% at the median follow-up of 30 months (range, 6-96 month). Complete or partial remission was achieved in 88% patients in IST group. All transplanted patients are in complete remission. The numbers of CD34+ and HAE-9+ BM cells were decreased in most of untreated AA patients (2-3-fold below normal). In control group the mean number apoptotic CD34+ cells was 1% and 81% cells were in G_0/G_1 phases of cell cycle. In more than 50% of AA patients before treatment $CD34^+$ progenitor cells were 2-3 fold more apoptotic. Also we revealed significantly greater proportion of $CD34^+$ cells in S/G2/M phases of cell cycle. No significant difference was found in cell cycle state of committed $HAE\text{-}9^+$ erythroid cells. The numbers of $CD34^+$ and $HAE\text{-}9^+$ cells rapidly increased after IST. Nevertheless increased apoptosis and cycling in $CD34^+$ cell population persisted even in patients with complete remission. $CD34^+$ cells compartment recovery was more complete in patients after alloBMT than after IST. The number of $CD3^+$ $CD8^+$ and $CD8^+DR^+$ lymphocytes significantly increased in BM during active phase of AA and returned to normal level at 6 month after ATG treatment. However the number of $CD8^+DR^+$ lymphocytes increased again in most of patients after 12 month follow-up. *Conclusion*: Modern treatment modalities provide hematological response and long-term survival in more than 80% of AA patients. Our data confirm that increased apoptosis and replicate stress in $CD34^+$ cell compartment with profound stem cells deficiency correlate with signs of T-cell activation process in AA. Assessment of hematopoietic reservoir and immune mediated pathology may provide additional information about remission status in AA patients.

1267

IL-12 AND IL-10 PRODUCTION BY DENDRITIC CELLS (DC) FROM PATIENTS WITH APLASTIC ANEMIA (AA)

S.H. Archuadze, E.A. Mikhailova, E.A. Sadovnikova, E.N. Parovichnikova, V.G. Savchenko

National Center for Haematology, MOSCOW, Russian Federation

DCs accomplish determining function in antigen-specific T-cell immune response and antigen-specific self-tolerance development. Moreover, they yield either Th1 or Th2 commitment of naïve lymphocytes due to immunoregulatory cytokines' (IL-10 and IL-12) production. As AA is characterized with increased Th1/Th2 ratio and Th1 mediated suppression of hematopoiesis, DCs might be responsible for HLA-DR2 restricted T-clone activation in AA patients. However, functional peculiarities such as IL-10 and IL-12 production by DC in AA patients still remain to be elucidated. Therefore, IL-12 and IL-10 secretion capabilities of DC generated from peripheral mononuclear cells (PMNC) of 23 AA patients before and after the immunosuppressive therapy (IST) were compared with DCs of 4 healthy donors (HDs). DC were derived from PMNC in the presence of GM-CSF and IL-4 for 5-7 days and further stimulated for 48 hours by exposition either to LPS or to CD40L expressing cells. Supernatants of DC cultures were tested by ELISA for IL-10 and IL-12 production, respectively. DC of 78,6% (17/23) of AA patients showed significantly lower levels of IL-10 production at baseline in comparison with control group data. 1st stage of IST resulted in a tendency of IL-10 production enhancement, though to lower amounts than that of HDs. Considerable increase in the level of IL-10 production by DCs of AA patients after durable IST correlated with achievement of partial or complete remission. Moreover, level of IL-10 production by DCs of AA patients in remission exceeded that of DCs of HDs (224 vs 166 pg/106cells, respectively). 36,4% (4/11) of AA patients exhibited increased baseline levels of IL-12 production by DCs compared to those of HDs. These patients appeared to be resistant to standard scheme of IST and required continuous IST including repeated courses of antithymocyte globulin and cyclosporine-A. However, significant diminution of IL-12 production by DCs of AA patients after I stage of IST was associated with favorable outcome. Patients that had shown low initial levels of IL-12 secretion by DCs comprised the best prognostic group. These data suggest that dysregulation of IL-10 and IL-12 production by DCs of AA patients might contribute to autocytotoxic T-clone expansion and consequently, to AA development.

1268

INHERITED BONE MARROW FAILURE SYNDROMES IN LEBANON. PILOT DATA FROM THE LEBANESE PEDIATRIC HEMATOLOGY/ONCOLOGY GROUP

R.A. Farah,[1] S.N. Laham,[2] C. Khayat,[3] P. Noun,[4] A. Kadri,[5] I. Dabbous,[6] A. Inaty-Khoriaty[7]

[1]*St Georges hospital, BEIRUT, Lebanon;* [2]*St Georges Hospital, BEIRUT, Lebanon;* [3]*Hotel Dieu de France Hospital, BEIRUT, Lebanon;* [4]*Lebanese Hospital, BEIRUT, Lebanon;* [5]*Tel Shiha Hospital, ZAHLE, Lebanon;* [6]*American University of Beirut Medical Ce, BEIRUT, Lebanon;* [7]*Nini Hospital, TRIPOLI, Lebanon*

Backgrounds. Inherited bone marrow failure syndromes (IBMFS) are rare disorders of the bone marrow with an increased risk of malignancy. Genetic features of these disorders are still being studied and could vary among different ethnic groups. Although some data is available from registries in the US and Europe, there is no such data from the Middle East and particularly from Lebanon. *Aims.* We aim to study the incidence, outcome and overall condition of patients affected with these disorders in Lebanon, as well as the genetic features of their families in order to establish a National IBMFS registry. *Methods.* Patients with the following diagnosis were included: Amegakaryocytic Thrombocytopenia, Diamond-Blackfan Anemia (DBA), Dyskeratosis Congenita, Fanconi's Anemia, Pearson's Syndrome, Severe Congenital Neutropenia, Shwachman-Diamond Syndrome, Thrombocytopenia Absent Radii (TAR). Data collection sheets were filled by the pediatric hematologist for each patient diagnosed with any of these disorders between 1970 and 2006. Data was later entered into an Excel workbook and statistical analysis was performed. *Results.* Fourty two (42) patients were identified so far. Fourteen had Fanconi anemia, nineteen had DBA, and six had severe congenital neutropenia, one had TAR syndrome, one had amegakaryocytic thrombocytopenia and one had Shwachman-Diamond Syndrome. Mean age was 10.2 years. At the time of data collection, 67% were alive and 33% were dead. Death was due to malignancy in 6 out

of 14 cases. DNA was available in 11 patients and was studied for possible mutations in the disease specific genes. Further results will be presented at the meeting. *Summary/Conclusions.* This is the first study looking at Inherited Bone Marrow Failure Syndromes in Lebanon. A registry has been created and is being updated constantly with new cases. A larger regional registry should be created with collaborative efforts and data should be compared among countries and then to registries in Europe and the USA in order to improve diagnosis and outcome of these patients and compare genetic determinants of these complex disorders.

1269

ALLOGENEIC TRANSPLANTATION IN ADVANCED STAGES OF CML IN THE THIRD MILLENIUM. IS THERE A DIFFERENCE ?

M. Markova, A. Vitek, H. Klamova, V. Valkova, M. Lukasova, J. Sajdova, D. Pohlreich, D. Sponerova, P. Cetkovsky

Institute of Haematology, PRAHA [2], Czech Republic

Although since 2001 thyrosine kinase inhibitors have substantially changed the therapeutic approach in CML (chronic myelogenous leukaemia), its advanced stages still remain an important problem, in which transplantation is considered. *Methods.* We evaluated retrospectively 36 patients transplanted between the years 1992-2005 in the advanced stages of CML (i.e. further than 1st chronic phase). 20 of them proceeded the treatment in the nineties (non-imatinib era)(group 1), while 16 patients were transplanted in the last 5 years (group 2). In these patients imatinib was used either before transplantation (12 pts, 4 of them progressed despite this treatment), or after the transplantation (6 pts). Differences in disease stage when entering the transplantation, transplant related mortality, overall survival, relaps rate and its response to further treatment were evaluated. *Results.* While in the group 1 there were only 25% of patients entering the transplantation in the re-achieved chronic phase, the number increased to 44% in the group 2. The median of overall survival was 1.5 months in the group 1 compared to 16 months in the group 2. There are 3 surviving patients (15%) in the group 1 and 11 (69%) in the group 2. There was an enormous 100 days mortality in the group 1 (3 pts, i.e. 65%) compared to absent 100 days mortality in the group 2. Remission was achieved in 6 patients in the group 1, (3 of them relapsed later) and in 14 patients in the group 2, (6 of them relapsed later). DLI (donor lymphocyte infusion) or next transplant was used as a relaps treatment in 2 patients in the group 1 and in 5 patients in the group 2. In 6 patients of the group 2 imatinib was used after transplant. *Conclusions.* More feasible and probably less toxic achievement of further chronic phase by chemotherapeutic combinations with imatinib, better supportive care, earlier detection of minimal residual disease or relaps by molecular techniques, the use of imatinib for post-transplant relapses and more frequent use of DLI might be the main contributors for better survival in patients transplanted in the advanced stages of CML.

1270

INFECTION TRANSMISSION DURING GRAFT IN STEM CELL TRANSPLANTATION SYSTEM OF PREVENTION

M. Blaha, P. Mericka, J. Maly, L. Jebavy, P. Zak, M. Cermanova, S. Filip, M. Blazek, R. Maly, V. Rehacek

Faculty Hospital, Medical Faculty, HRADEC KRALOV, Czech Republic

Backgrounds. The possibility of infection transmission by transplantation of cryopreserved blood stem cell concentrates is well known. For this reason EBMT (European Blood and Marrow Transplantation Group) and ISHAGE (International Society for Haemotherapy and Graft Engineering) standards include a panel of serological tests to be performed in donors and patients with the aim to lower the likelihood of infection transmission. *Aim*: In the submitted paper attention is focused on danger of infection transmission by infusion of cryopreserved peripheral blood progenitor cells or bone marrow to the patient and/or cross contamination of stored grafts. *Methods.* After our preliminary investigations published 3 years ago the study was performed on a group of 35 related donors for allogeneic transplantation and 152 pts (mal.lymphomas, multiple myel., leukemias, solid tumors, amyloidosis). They were tested before the peripheral blood stem cell or bone marrow harvest according to the standards of EBMT and ISHAGE-Europe: retroviruses (HIV,HTVL), hepatitis (A,B,C), herpes viruses (CMV, EBV, VZV, HSV), lues, toxoplasmosis. *Results.* No laboratory signs of active infection were found in 22 donors (62,85%) and in 91 patients (59.9%). The active infection from herpes viruses was the most common - in patients 50, in donors 21. Hepatitis B was found in only two cases. *Conclusion*: We can conclude that the rate of clinically unsuspected (but dangerous) infections in donors and patients remains relatively high in spite of the fact that the system of donor search and the whole transplantation procedure have improved in the last years. We confirmed that the developed system of safety assurance is extremely important and that the whole palette of preventive tests according to EBMT and ISHAGE remains fully justified.

Funding: Supported by the grants NR/8505-3, NR/8062-3, MZO 00179906, MSM 0021620820, NR/9103-4, NR/9118-3.

1271

EVOLUTION OF PATIENTS WITH ESSENTIAL THROMBOCYTEMIA (ET) TREATED WITH HYDROXIUREA (HU) AND α - INTERFERON (IFN)

A. Colita,[1] A.R. Lupu,[1] N. Berbec,[1] G. Mocanu,[2] S. Angelescu,[2] D. Barbu,[2] C. Vlaicu,[2] S. Crintea,[2] O. Ciocan,[1] M. Closca,[2] A.M. Vladareanu,[3] H. Bumbea,[3] D. Mut Popescu[1]

[1]*Coltea Clinical Hospital/UMF Carol Davil, BUCHAREST, Romania;* [2]*Coltea Hospital, BUCHAREST, Romania;* [3]*University Hospital/ UMF Carol Davila, BUCHAREST, Romania*

Backgrounds. Essential thrombocytemia (ET) is a clonal myeloproliferative disease characterized by increased number of platelets, megakariocytic hiperplasia and tendency to thrombosis and/or hemorrhage. The major aim of therapy is preventing thrombotic and hemorrhagic complications using cytoreductive agents that do not increase the risk of progression to acute leukemia or myelofibrosis. *Aim of the study*. to compare the results of the therapy with HU versus IFN in ET pacients. *Patients and Methods.* 72 pacients with ET followed between June 1999 - July 2005; median age 59,5 years (range 33 - 87 years); M/F: 32/40. Diagnostic criteria were those of the PVSG. 44 pacients (median age 62 years) received HU, and 28 pacients (median age 51 years) received IFN. HU dosis varied according to platelet counts between 500 and 1500 mg/d. IFN dosis was 9 MU/week. The purpose of therapy was to maitain platelet counts below 600.000/cmm and to prevent thrombotic si hemorrhagic complications. Patient with high risk for thrombosis received aspirine (75 mg/zi). *Results.* Pacients treated with IFN presented a 90% response rate and those receiving HU had a 75% response rate. The reduction of platelet counts below 600.000/cmm was faster in the IFN group versus HU group (average 4 weeks vs 10 weeks respectively). The level of platelets during the treatment was maintained constantly arround 400.000/cmm with IFN whereas in the HU group it was arround 600.000/cmm. Thrombotic complications occurred more often in the IFN group - 8 cases (28,5%) with predominance of arterial thrombosis - 6 cases. In the HU group the incidence of thrombosis was 18,18% (8 cases - 3 arterial thrombosis). The treatment with HU was better tolerated ‘6 cases with reversible leucopenia. The therapy with IFN was worse tolerated - 4 patients abandoned the treatment because of general simptoms. *Conclusions.* IFN was more effective in assuring a rapid and constant decrease of platelet number, but in the HU-treated group there was a lower incidence of thrombotic complications.

1272

CHRONIC MYELOPROLIFERATIVE DISORDERS: USE OF WHOLE BLOOD PLATELET LUMI-AGGREGOMETRY (WBPA) TO OPTIMISE ANTI-PLATELET THERAPY IN PATIENTS WITH PLATELET HYPERACTIVITY

A. Manoharan, R. Gemmell, T. Hartwell

St. George Hospital, SYDNEY, Australia

Twenty seven patients with chronic myeloproliferative disorders and in vitro evidence of platelet hyperactivity on WBPA studies (Br J Haematol 199;105:618) were commenced on anti-platelet therapy comprising aspirin, clopidogrel and/or odourless garlic and the studies were repeated to assess the efficacy of the therapeutic agents. Only eight patients showed clear evidence of anti-platelet effect (inhibition of aggregation with arachidonic acid; aggregation and disaggregation with ristocetin), whilst receiving the standard low dose (100 mg/d) aspirin therapy. Thirteen patients required a higher dosage of aspirin and/or an additional anti-platelet agent to achieve therapeutic efficacy. Lumi-aggregometry also proved useful to optimise therapy in the six patients who received clopidogrel (reduction in response to ADP) or odourless garlic (change of overall platelet function from hyper-activity to normal or hypo-activity), because of aspirin intolerance. *Conclusion*. Our experience suggests that WBPA studies will not only enable selection of patients who will benefit from anti-platelet therapy but also assess the efficacy of such therapy.

1273

PREVALENCE OF THE ACQUIRED MUTATION V617F ON THE JAK2 GENE ON THE DIFFERENT MYELOPRILIFERATIVE DISORDERS, AND ITS CONTRIBUTION TO A CORRECT DIAGNOSIS

C.E. Lopez Jorge,[1] J. Lopez Brito,[1] P. Martin,[1] M.T. Gomez Casares,[1] J.D. Gonzalez San Miquel,[2] A. Suarez,[2] G. Santana,[1] T. Ramirez,[1] M. Perera,[3] H. Luzardo,[1] L. Guerra,[1] T. Molero

[1]Hospital Universitario Doctor Negrin, LAS PALMAS DE GRAN CANARIA, Spain; [2]Hospital Universitario Insular, LAS PALMAS DE GRAN CANARIA, Spain; [3]HOSPITAL UNIVERSITARIO DOCTOR NEGRÍN, LAS PALMAS DE GRAN CANARIA, Spain

Backgrounds. The molecular basis for Ph- Chronic Myeloid Disorders (CMPD) remain widely unknown, although nowadays, mutations on the *JAK2* gene are thought to play a role in its pathogenesis. The Janus Kinase 2 gene (*JAK2*) is a tyrosin kinase involved in the transduction of cell proliferation stimuli. Unlike healthy patients, the *JAK2* point mutation, 1849G→T which produces the substitution of Phe for Val (V617F) on the resulting protein, has been identified on many CMPD cases. *Aims.* To study the prevalence of the point mutation V617F on *JAK2* on patients suffering from CMPD, and to determine its potential contribution for the classification and final diagnosis in these cases. Methods. A total of 99 patients were studied, which distributed as follows: 16 cases of reactive myeloid cytoses, 14 suspected CMPD, and 69 confirmed CMPD. Within the latter group there were: 5 chronic idiopathic myelofibrosis (CIM), 14 Policitemia Vera (PV), 28 essential thrombocythemia (ET), and 22 mixed CMPD. The screening for the *JAK2* mutation was performed on samples obtained from bone marrow aspirates, or peripheral blood, using PCR according to E. Joanna Baxter technique (*Lancet 2005*). Results. From the total of patients studied (99), 53 were JAK2+ (53.5%), and distributed as follows: 3 on the group diagnosed as reactive myeloid cytoses (18.7%), 4 on the non confirmed CMPD (28.6%), and 46 on the CMPD (66.7%). The *JAK2*$^+$ mutation's prevalence on the different subgroups of CMPD was: 15 (53.6%) of 28 for ET, 13 (92.8%) of 14 cases of PV, 3 (60.0%) of 5 cases of CIM, and 15 (68.2%) of 22 patients with mixed CMPD. *Conclusions.* 1) Allele-specific PCR is an effective method for the detection of JAK2 mutation with no need of a mutation screening. 2) Inside the CMPD, the highest percentage of *JAK2*$^+$ patients belonged to the PV group. 3) The percentage of *JAK2*$^+$ patients on the mixed CMPD is also high, which could mean that among these patients some PV cases could still remain incorrectly diagnosed. 4) The screening for the JAK2 mutation was useful to diagnose CMPD on patients which had been previously diagnosed as reactive myeloid cytoses. This derives, in part, because CMPD are frecuently difficult to diagnose due to the considerable clinico-pathologic overlap with reactive cytoses.

1274

PREVALENCE OF THE ACTIVATING *JAK2* TYROSINE KINASE MUTATION *V617F* IN TAIWANESE PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

H.C. Hsu,[1] H.S. Wu,[2] Y.C. Hon,[1] C.C. Wang,[1] C.F. Yang,[1] P.M. Chen,[1] C.H. Lieu[2]

[1]Taipei-Veterans General Hospital, TAIPEI, Taiwan; [2]National Yang-Ming University, TAIPEI, Taiwan

Background. The *JAK2 V617F* mutation has been recently reported in patients with polycythemia vera(PV) and a proportion of patients with essential thrombocythemia(ET) and myelofibrosis with myeloid metaplasia(MMF). This acquired point mutation constitutively activates *JAK2* tyrosine kinase and is believed to underlie growth factor hypersensitivity displayed by hematopoietic progenitors in these disorders. *Patients and Methods*. In this study, allele-specific PCR (ASPCR) was performed with a primer pair common to both wild-type and mutant alleles. We amplified a JAK2 exon 12 fragment from peripheral blood leukocyte from 96 patients by ASPCR followed by digestion with BsaXI restriction endonuclease (PCR-RFLP). The *JAK2V617F* mutation abolishes a BsaXI restriction site present in the wild-type sequence and generates a different band pattern. *Results*. *JAK2* mutation could be detected in 25 of 31 PV patients (80.6%), 23 of 38 ET patients (60.5%), 2 of 6 MMF patients (33%), none of 11 MDS patients and 10 patients with other diseases. There is no significant difference between JAK2 mutation- positive and 'negative patients in the age, peripheral blood counts, creatinine and antecedent cancer history. The thrombotic or cerebral bleeding complication occurred in 13 out of 48 patients with JAK2 mutation (+), but only 4 of 25 patients with JAK2 mutation (-) (27% vs. 16%; p=0.44). *Conclusions*. *JAK2 V617F* mutation can be frequently detected in the Taiwanese patients with myeloproliferative disorders as in the western patients, which should be used as a diagnostic tool in the future routine hematological practice.

1275

EPO-R MUTATIONS IN FAMILIAL CONGENITAL POLYCYTHEMIA VERA

L. Bourantas,[1] A. Chatzikyriakidou,[2] A. Dasoula,[3] E. Hatzimichael,[1] St. Tsiara,[1] M. Syrrou,[3] K. Bourantas,[1] I. Georgiou[2]

[1]Haematology Clinic, IOANNINA, Greece; [2]Laboratory of Genetics Unit, IOANNINA, Greece; [3]Laboratory of General Biology, IOANNINA, Greece

Backgrounds. Primary familial congenital polycythemia (PFCP) is a rare myeloproliferative congenital and dominant disorder. It is caused by inherited defects in hypoxia sensing or by inherited vital defects in red blood cell precursors that cause augmented responsiveness to Epo. These facts result in isolated proliferation of the erythroid progenitor cells and in erythrocytosis. The Epo-receptors (Epo-R) is lexated on the surface of erythroid cells. The Epo-R gene is situated on chromosome 19. Genetic changes of the Epo-R gene have been related to the pathogenesis of PFCP. Specifically, twelve mutations in patients with PFCP have been recognized. Aim of this study was to investigate the presence of Epo-R mutations in patients with PFPC. *Methods.* We studied eight families (20 individuals) with PFCP of Greek origin. All individuals had Ht>53% and their age range was between 16 and 58 years. Genomic DNA was extracted from peripheral blood lymphocytes, according to standard procedures. SSCP and sequencing analysis was performed to detect mutations in exon VIII, of the Epo-R gene that previously has been related to PFCP. *Results.* No mutation was identified in our patients which could underlie the molecular defects of the PFCP. *Conclusions.* Our results can lead to the conclusion that the molecular cause of familiar polycythemias in Greek patients cannot be attributed to sequence alterations in exon VIII of the Epo-R gene.

1276

HLA ASSOCIATIONS WITH CHILD'S AND ADULT'S ACUTE LYMPHOBLASTIC LEUKEMIA

E. Khamaganova, L. Murashova, O. Korovina, Y. Zaretskaya

Research Center for Hematology, MOSCOW, Russian Federation

Background. Acute lymphoblastic leukemia (ALL) has two rising of morbidity: in children of 2-4 years and in adults after 50 years. The aim of our study was to identify the associations between HLA and ALL in children and in adults in our population.

Table 1. DRB1 frequencies in children and adults with ALL.

	Children with ALL n=26 I	Adults with ALL n=42 II	Controls n=328 III	I-II	I-III	II-III
DRB1*01	7.7	11.9	26.2	p>0.05	p<0.01	p<0.05
DRB1*07	46.2	21.4	26.8	p<0.05	p<0.05	p>0.05
DRB1*11	34.6	38.1	22.6	p>0.05	p>0.05	p<0.05

Methods. HLA-A, -B, -Cw, -DRB1 typing was done in 26 children with ALL (the median patient age was years 5.6; range, 1- 13 years) and in 42 adult patients with ALL (the median patient age was 32.6 years; range, 16-52 years). 328 healthy donors of blood components (the median age was 29.9 years; range 18-59 years) were control group. The HLA frequencies were counted and compared by exact Fisher's test.The strength of association between HLA and disease was determined by the evaluation of relative risk (RR). *Results*. Children with ALL had significantly increased frequency of *DRB1*07* (46.2% vs. 26.8% in control group, see the table below), RR of child's ALL for DRB1*07 carriers was 2.3. Adults with ALL had significantly increased frequency of *DRB1*11* (38.1% vs. 22.6% in control group). RR of adult's ALL for *DRB1*11* carriers was 2.1. The frequency of *DRB1*01* was significantly decreased in both groups: in children with ALL (7.7%) and in adults with ALL (11.9%) in comparison with controls (26.2%). RR of child's and adult's ALL for *DRB1*01* carriers in our population was 0.23 and 0.38 respectively. In conclusion, it seems that DRB1 gene may be involved in predisposition and resistance to ALL development both in children and in adults.

1277

INTERMEDIATE ANALYSIS OF THE SPANISH QUIT REGISTRY FOR PATIENTS WITH ACUTE LEUKEMIAS AND NON-HODGKINS LYMPHOMAS TREATED WITH INTRATHECAL CHEMOTHERAPY

J.M. Sancho, J.M. Ribera, N. Alonso, G. Deben, A. Fernandez de Sevilla, P. Fernandez-Abellan, R. Arranz, E. Sanchez, C. Nicolas, M. Blanes, L. Vazquez, J. Arias, J.A. Garcia-Vela, E. Abella, A. Rueda, E. Conde

QUIT study, BADALONA, Spain

Background and Aim. CNS involvement in pts diagnosed with hematological malignancies is an unfrequent complication that carries poor prognosis. The indication and the schedules of prophylaxis and treatment of CNS involvement in AL and NHL are not homogenous within countries and within the same country. The aim of the QUIT study was to report the practices of CNS prophylaxis and treatment in patients with AL and NHL in Spain. *Methods*. Prospective study conducted from June to December 2005. Adult pts (≥18 yr.) diagnosed with hematological malignancies who received IT chemotherapy as CNS prophylaxis or treatment were consecutively included through online registration. Results. 242 pts from 27 hospitals were included. Mean (SD) age 48 (16) yr. and 133 (55%) males; 142 had AL and 100 NHL. 1. AL patients: 85 ALL and 57 AML. CNS therapy: 17 cases, 11 at diagnosis and 6 as relapse (5 isolated, 1 combined). IT therapy consisted of triple IT therapy (TIT, MTX+ARAC+Hydrocortisone) in 14 and liposomal depot cytarabine in 3 pts. CNS prophylaxis: 125 patients and consisted of TIT in 104 (83%), and MTX IT in 19 (15%), IT ARAC in 1 (1%) and 1 IT liposomal depot cytarabine (1%) L. No cranial irradiation either for prophylaxis or therapy was given in any case. 2. NHL patients: 56 diffuse large B-cell, 18 Burkitt's, 5 follicular, 5 mantle cell, 10 T cell, and 6 other subtypes; stage IV 70%, B symptoms 52%, bulky disease 31%, extranodal involvement 79% (bone marrow 43%) and >1 extranodal site 44%, increased LDH levels 64%, IPI score 3 68%. CNS therapy: 24 pts, 16 at diagnosis and 8 as first (5) or subsequent relapses (3). CNS therapy consisted of TIT in 15/24 patients (62%), IT liposomal depot cytarabine in 8/24 (33%) and MTX IT in one (4%). CNS prophylaxis was given to 76 pts, the main reasons for administration were extranodal involvement (85%), aggressive histology (66%), high LDH levels (46%), IPI score ≥3 (31%), bulky disease (26%) and HIV infection (10%). IT prophylaxis consisted of TIT in 88% followed by MTX IT in 9% and IT liposomal depot cytarabine in 3%; Cranial irradiation was administered in 3 cases (2 as therapy an 1 as prophylaxis). *Conclusions*. In clinical practice in Spain the patterns of CNS prophylaxis and therapy for AL are homogeneous. For NHL there is heterogeneity of indications for CNS prophylaxis. TIT was the most frequent schedule for CNS prophylaxis and therapy in AL and NHL. It is of note the administration of new drugs i.e.: liposomal depot cytarabine for CNS therapy and prophylaxis in NHL and AL, and the lack of use of cranial irradiation.

1278

COMPARISION OF METHODS FOR DETERMINIG ζ-CHAIN ASSOCIATED PROTEIN 70 (ZAP-70) EXPRESSION IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL)

K.G. Giannopoulos,[1] A. Bojarska-Junak,[2] M. Kowal,[3] A. Dmoszynska,[3] J. Rolinski[2]

[1]*Medical University of Lublin, LUBLIN, Poland;* [2]*Dept. of Clinical Immunology, LUBLIN, Poland;* [3]*Dept. of Hematooncology, LUBLIN, Poland*

Background and Aims. ζ-chain associated protein of 70kDa (ZAP-70) is the most promising surrogate marker for the IgVH mutation status. ZAP-70 is a signaling molecule from Syk/ZAP-70 protein kinase family normally utilised by T and NK cell and abnormously expressed in B-CLL cells. Expression of ZAP-70 protein in B-CLL detected by flow-cytometric analysis, correlated with IgVH mutational status, disease progression as well as survival and revealed even better prognostic value compared to IgVH mutation. Crespo et al. proposed the method of ZAP-70 detection by flow cytometric test. Recently several novel monoclonal antibodies appeared on market. In this paper we compared different methods of determining ZAP-70 expression in B-CLL. We wanted to find most clinically relevant and easy assay to determine ZAP-70 expression in B-CLL. *Methods.* We compared different clones of monoclonal antibodies against ZAP-70 with direct and indirect staining, ZAP-70 expression utilizing whole blood protocol and peripheral mononuclear cells isolated from whole blood and additionally the use of different reagents for permeabilization. *Results and Conclusions.* Basing on results obtained during this study in 45 previously untreated B-CLL patients we can recommend use of anti-ZAP-70 PE, clone 1E7.2 monoclonal antibodies utilizing whole blood protocol as an easy method that brings completely compatible results to the original method proposed by Crespo *et al*.

1279

EXPRESSION OF *ZAP-70*, *CD38* AND *IGVH* MUTATIONAL STATUS AS PREDICTORS OF TREATMENT IN BINET STAGE A CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

M.J. Terol, I. Benet, I. Marugan, P. Eroles, A. Teruel, M. Tormo, C. Solano

Hospital Clinico, VALENCIA, Spain

Backgrounds. a combination of Zap-70/CD38 expression has been proposed as a predictor of treatment in CLL patients by several authors. *Aim:* to analyse the predictive value of this model in a series of 73 patients consecutively diagnosed of B-CLL at our institution from 1997 until 2005, whose all three variables were available. *Methods.* Zap-70 and CD38 expression was analysed by flow cytometry and IgVH mutational status by direct sequencing with 98% cut off. All 73 patients were in Binet stage A. Median age was 66 years (34 to 85), male sex 40 (55%). ZAP-70 and CD38 expression cut off were 20% for both antigens. Median follow-up was 56 months (6,7 to 134).

Table 1.

Variable		*N(%)*	*Time to treatment*	*p*
CD38	<20%	48 (66)	NR	0.013
	≥20%	25 (34)	42.9	
ZAP-70	<20%	44 (60)	NR	0.013
	≥20%	29 (40)	48.3	
IgVH	mutated	40 (55)	NR	0.015
	unmutated	33 (45)	43.7	
ZAP-70/IgVH	positive/unmutated	21 (29)	48.3	0.007
	discordant	20 (27)	42.9	
	negative/mutated	32 (44)	NR	
CD38/IgVH	positive/unmutated	17 (23)	42.9	0.0038
	discordant	24 (33)	43.7	
	negative/mutated	32 (44)	NR	
CD38/IgVH	positive both	20 (27)	39	0.014
	discordant	14 (19)	NR	
	negative both	39 (53)	NR	

NR: not reached.

Results. All 3 variables provided significant prognostic information with respect to the need of treatment. An intermediate prognostic group was identified for discordant cases. The predictive value of the three dual combinations is detailed in the table above. Concordant positive expression of CD38/ZAP-70 identifies an aggressive group of patients with the shorter time to first treatment (39 months versus not reached) and the lower percentage of discordant cases (19%). CD38/ZAP-70 positive expression predicted an unmutated status of IgVH gene in 75% of the cases whereas negativity for both antigens predicted a mutated status in 85% of cases. In the IgVH mutated group (n=40), 3 out of 11 patients treated were discordant cases who co expressed both antigens. Conclusion: in Binet A stage CLL patients, CD38/ZAP-70 positive expression allows to identify a group of patients with a shorter time to first treatment (39 months) in concordant cases without the knowledge of the mutational IgVH gene status

1280

LONG-TERM RESULTS OF THALIDOMIDE IN REFRACTORY AND RELAPSED MULTIPLE MYELOMA WITH EMPHASIS ON RESPONSE DURATION

M.T. Cibeira,[1] L. Rosinol,[2] O. Salamero,[2] G. Gutierrez,[2] M. Torrebadell,[2] J. Esteve,[2] J. Blade,[2] E. Montserrat[2]

[1]*Hospital Clnic, IDIBAPS, BARCELONA, Spain;* [2]*Hospital Clinic, IDIBAPS, BARCELONA, Spain*

Background. Thalidomide administered as a single agent produces a response rate of about 40% in patients with refractory or relapsed multiple myeloma (MM). However, the data on the duration of such responses is limited. *Aim*. The aim of our study was to determine the

quality and duration of responses to thalidomide in patients with refractory or relapsed MM. *Patients and methods*. Forty-two consecutive patients (22M/20F, median age: 61 years) with refractory (20) or relapsed (22) MM were given thalidomide as single agent at our institution from November 1999 to December 2003. Most of the patients (70%) had previously received 2 or more lines of therapy, and 38% had undergone autologous stem cell transplantation (ASCT). The drug was initially administered at a daily dose of 200 mg and later increased, depending on tolerance, by 100 to 200 mg every two weeks up to a daily dose of 800 mg. In responding patients, the dose of thalidomide was thereafter progressively tapered to a maintenance dose of 100 mg/day. No prophylactic anticoagulation was given. *Results*. Eighteen patients (43%) responded to thalidomide: 11 minimal responses (MR) and 7 partial responses (PR) according to the EBMT criteria. The median time to response was 3 months and the median duration of therapy in responding patients was 9 months. The reasons for discontinuing thalidomide in responding patients were: toxicity in 10 cases, progression in 4, and death due to pneumonia with respiratory failure in 2. In 2 additional patients treatment was stopped at the time when they were intensified with ASCT. The toxicity mainly consisted of peripheral neuropathy and fatigue. At the time of this analysis, all responding patients had progressed except one who remains in continued stable PR for more than six years after starting thalidomide therapy and for 3.5 years after thalidomide discontinuation. The median time to progression was 15.6 months (range: 1.3-70+), with a trend towards a longer duration for patients who achieved PR vs. MR (21.2 vs. 11.2 months, p=0.11). The median duration of response was 12.4 months (range: 0.3-67+) (17.2 months for PR vs. 9.7 months for MR, p=0.11). *Conclusion*. These results show that the effect of thalidomide in refractory/relapsed MM can be sustained, particularly in patients who achieve a good response, and support the investigation of this drug as maintenance therapy.

1281

THCY AND VASCULAR DISEASE. HOMOCYSTEINE AND VASCULAR DISEASE. IS THERE ANY RELATION AFTER ALL?

A.Skordilaki, F. Samiotaki, N. Tsagarakis

General Hospital Hania Crete Greece, HANIA, Greece; Transfusion Service-General Hospital of Chanea, Krete,Greece

During the last years, after the completion of several epidemic studies, it is supported by many researchers that homocysteine is another risk factor of vascular disease. The aim of our study was to evaluate the homocysteine's plasma levels in patients who suffered from coronary disease or a cerebrovascular accident. The patients were categorized into 3 groups. Group A: 130 healthy volunteer blood donors, of whom 108 were men with mean average age 35.7 years old (19-57) and 22 women with mean average age 33.95 years old (22-55). Group B: 68 with cerebrovascular accident, confirmed with brain CT or MRI, of whom 31 were men with mean average age 52.6 years old (31-75) and 37 women with mean average age 51.21 years old (23-67). Group C: 34 patients with an acute coronary syndrome, of whom 26 were men with mean average age 50.5 years old (35-61) and 8 women with mean average age 44.6 years old (34-55). In groups B,C patients with other risk factors, such as hypertension, diabetes mellitus, hyperlipidemia etc, were not included. The homocysteine measurement was performed with Elisa (ABBOT-AXSYM) and the blood test was done, in all 3 groups, under the same circumstances. In the following table see the results.

Table 1.

Group A	*Group B*	*Group C*
Men 11.4±5.8μmol/L	Men 11.33±2.94μmol/L	Men 11.2±2.4μmol/L
Women 8.98±2.13	Women 8.81±2.52	Women 14.1±7.5

If we exclude those women with cardiovascular disease who had higher homocysteine's plasma levels from women of the other groups, significant differences among the levels of the others were not noticed. Thus many questions remain to be answered before tHcy is finally considered as a risk factor of cardiovascular disease or stroke. Many more studies are needed before we are able to focus our attention in therapy and prevention.

1282

ANALYSIS OF THE EFFECTIVE AGENTS IN DEVELOPMENT OF FACTOR INHIBITOR IN HEMOPHILIC PATIENTS EVALUATION OF 445 PATIENTS IN CENTRAL PART OF IRAN

M. Mojtabavi Naini, F. Derakhshan, S. Zolfaghari Anaraki, F. Makarian Rajabi, H. Hoorfar, R. Derakhshan

Isfahan University of Medical Sciences, ISFAHAN, Iran

Backgrounds. In hemophilia the development of inhibitors is a serious problem. Inhibitors reduce the efficacy of haemostatic treatment and clearly cause additional morbidity. Understanding the effective agents in arising factor inhibitors could be helpful in management of hemophilic patients. *Aim:* To evaluate the development of inhibitors and analysis the effective agents in it, in patients with hemophilia. *Method*: A comprehensive study underwent in Hematology-Oncology Department- Isfahan University of Medical Sciences. All hemophilic patients (445 patients) underwent frequent testing for inhibitors and the development of an inhibitor was defined by a titer > 0.5 Bethesda units (BU)/ml in any sample. Clinical history, Laboratory and treatment data of all patients were studied in January 2006. *Results.* from 401 men and 44 women with factor deficiency with Mean±SD age of 23.25±13.15, 27 patients (6.06%) showed factor inhibitor. The mean duration between diagnosis of disease and inhibitor arising was 15.26 years. From them 26 had Factor (F) VIII deficiency who were 7.6% of patients with FVIII deficiency, and one had FIX deficiency. According to forward stepwise logistic model (with percentage correct of 95%) treatment with factor concentrates with 2.9 times, and FVIII deficiency with 12.7 times chance correlate with factor inhibition. Other agents like, severity of disease, blood group and age of patients do not enter in the model. In this study 12.4% of patients who used factor concentrates, develop factor inhibitor (p=0.000). *Conclusion*: These data provide estimates of the rate of inhibitor in factor deficiencies. Beside several advantages of factor usage in treatments of hemophilic patients, the more chance of coloration between inhibitor development and it should be noticed.

1283

MANAGEMENT OF LIFE THREATENING SEVERE HEMORRHAGES AND UNSAFE INTERVENTIONS IN NON-HAEMOPHILIAC CHILDREN BY RECOMBINANT ACTIVATED FACTOR VIIA (RFVIIA)

B. Antmen, G. Leblebisatan, I. Sasmaz, S. Yavuz, D. Yildizdas, Y. Kilinc

Cukurova University Medical Faculty, ADANA, Turkey

The literature on the use of recombinant factor VIIa (rFVIIa) that was initially used in hemophiliac patients with inhibitors, for hemorrhages that cannot be managed with conventional methods or operations that cannot be performed safely is increasingly growing. Here we present a group of non-hemophiliac patients with hemorrhagic problems or hemorrhage risk for some interventions that were successfully resolved with the use of rFVIIa. The patient group was composed of 20 patients with different disorders resulting in similar results as hemorrhage or hemorrhage risk. Most of the patients were diagnosed as primary or secondary liver disorders. The remaining cases were patients with leukemia, sepsis, intracranial hemorrhage, and burn. Same of the patients had multiple problems like a patient with liver disorder and intracranial hemorrhage or a leukemic patient with sepsis and DIC. rFVII had been administered to the patients at dosage between 70-150 microg/kg up to 6 doses with 2-3 hours intervals. All of the patients had benefited from the use of rFVIIa even though some of them ceased as a result of primary disease. As in our experiences, rFVIIa can be safely used in high-risk patients with a history of severe hemorrhage, for whom no improvement can be achieved in the hemostasis tests. We conclude that rFVIIa is effective in the control of life threatening hemorrhage in pediatric patients.

1284

HEMARTHROSIS AS COMPLICATION OF SUPERWARFARIN POISONING

P.E. Makris,[1] P. Kotsaftis,[2] I. Avramidis,[2] A.K. Boutou,[2] M. Apostolopoulou,[2] A. Mirou,[2] M.P. Makris,[3] F. Girtovitis[2]

[1]*Aristotle University of Thessaloniki, THESSALONIKI, Greece;* [2]*Thrombosis and Haemostasis Unit, THESSALONIKI, Greece;* [3]*Thrombosis and Haemostasis Unit, THESSALONIKI, Greece*

Poisoning with long-acting anticoagulants is known to lead to disturbance of hemostasis. Such haemorrhagic complications, like the ones after poisoning with rodenticide, are often difficult to identify. To present, for the first time in literature, a case of haemarthrosis as a haemor-

rhagic complication of superwarfarin poisoning. A 67-year-old man was admitted to our clinic with melaena, epistaxis and haemarthrosis in his left knee. He received portion of rodenticide substance 15 days ago. 2 days after the reception he was hospitalized for 6 days with melaena and epistaxis. Screening tests of haemostasis revealed undetermined PT (INR) and aPTT. Initially, the patient was treated with 4 units of fresh frozen plasma (FFP) and 3×10 mg of Vitamin K iv per day, for 6 days. For further investigation he was admitted in our unit, where, during the first 7 days of hospitalization, had also melaena. We administrated supporting treatment (PPIs and 6 units of red cells) and we successfully treated epistaxis with topical haemostasis. Haemarthrosis was treated with FFP (4 unitsX2 per day, for two months). Initial screening; 1st day: INR=7.15, PT=60.6'. aPTT=79.9', Hct=28%. 2nd day: INR=15.2, PT=107.8', aPTT = 95', Hct= 23.5%. After the third day, values of INR varied from 2.04 to 4.78. PT from 15.5' to 44.7' and aPTT from 35' to 75.7'. After the 11th day the Hct remained stable at 34%. During the 23th day we administered 1X10 mg Vitamin K iv and three days later 3X10mg iv. Screening test at 26th day showed: INR=1.19, PT=15.2', APTT=37.5', Hct=32%. Immunological and biochemical tests, levels of electrolytes, complete study of haemostasis, microscopic examination of excrements for fat and undigested fibers, tests for viruses and complete study of liver function (for latent hepatic insufficiency) were performed. We found very low levels of vitamin K dependant factors (II, VII, IX, X, protein C and S), which were normalized after the administration of FFP and Vitamin K. Levels of the rest of the factors were normal. Erosions in antrum, bulb, and 2nd section of duodenum were revealed endoscopically, while the psychiatric estimation revealed a disturbed personality. Acquired disturbances of haemostasis after poisoning with superwarfarin (rodenticides) substances were described in several cases and have often led to death. This is why a long duration treatment and follow-up are required. Haemarthrosis as a complication of superwarfarin poisoning is presented for the first time in literature.

1285

PLATELETS COLLECTED BY MULTICOMPONENT APHERESIS WITH TWO DIFFERENT DEVICES: ACTIVATION MARKERS AND FUNCTION IN AGGREGOMETRY DURING SEVEN DAYS OF STORAGE

K. Schallmoser, S. Macher, S. Macher, S. Sipurzynski, K. Rosskopf, G. Lanzer

Medical University of Graz, GRAZ, Austria

Introduction. The collection of red blood cells (RBC) and platelets (plt) by multicomponent collection (MCC) is a mean to reduce donor exposure of polytransfused patients. We compared plt activation and plt function parameters in platelet concentrates (PC) collected by two different devices during 7 days storage to estimate a possible association between level of activation and collection modality as well as maintained platelet function. *Materials and Methods.* Fifteen donors, each with two donations, were included in our study. For each donor we used the TRIMA Access (Gambro BCT) (T-PC) and AMICUS (Baxter) (A-PC) with an interval of at least two months in between. PC were stored under agitation at 22±2°C and were tested on day 0, 2 and 7 for plt activation markers CD62P, CD63 and thrombospondin binding (TSP) by flow cytometry (FACSCalibur, Becton Dickinson). Additionally, aggregometry was performed using collagen as agonist (BCT, Dade Behring) and maximum aggregation (MA,%) and maximum velocity of aggregation (Vmax, mE/min) was measured. *Results.* Per apheresis a double PC and one unit of packed RBC (250ml, hct 80%). were collected. Mean plt concentrations in T-PC (1,407±103×10^9/L) and A-PC (1,291±228×10^9/L) were comparable (p=0.09). As shown in the table we found statistically significant higher percentages of CD62P- (d0 and d2), CD63- and TSP- (d0, 2 and 7) expressing plt in A-PC compared to T-PC.

Table 1.

	CD62p %	*CD63p* %	*TSP* %	*MA* %	*Vmax* mE/sec
d0 T-PC	17.3±6.1	19.2±9.4	11.5±5.1	86.7±5.3	37.6±13.9
A-PC	62.6±18.5**	36.2±16.5**	25.2±7.1**	68.9±15.6**	25.1±14.1*
d2 T-PC	32.6±12.6	34.9±12.5	17.3±6.1	81.7±3.6	28.1±12.3
A-PC	60.7±23.6**	62.1±20.9**	26.0±9.2*	56.7±20.5**	16.3±9.9*
d7 T-PC	67.5±8.7	46.3±8.8	21.4±7.5	34.1±27.6	10.4±8.7
A-PC	75.2±12.1	69.2±19.2**	28.9±9.6**	16.3±24.4	5.9±10.7

In contrast the disposition of platelets to get activated by collagen in aggregometry and the velocity of aggregation was significantly lower in A-PC on d0 and d2 compared to T-PC (values as mean±SD; * p<0.05; ** p<0.01).*Conclusion*: Standardised PC-collection in MCC is effective and well tolerated as expected and there are no differences in storage parameters in comparison to platelet collection only. Preparation of plt by the AMICUS cell separator leads to a higher activation level of plt and therefore to a reduced ability to be further activated to aggregate in vitro. This fact may be due to the different plt collection modality. In the AMICUS device plt are centrifuged highly concentrated until the end of apheresis and resuspended in plasma afterwards contrary to the TRIMA Access, where plt rich plasma is collected outside the cell separator. However, the in vivo relevance of our findings has to be evaluated in clinical observations.

1286

BACTERIAL CONTAMINATION OF APHERESIS PRODUCT FOR AUTOLOGOUS PERIPHERAL BLOOD TRANSPLANTATION AND THEIR CORRELATION WITH POST-TRANSPLANT BEHAVIOUR

A.M. García-Hernández, M.J. Majado, V. Sánchez-Ibáñez, C. González-García, P. Rosique, V. Martínez-Sánchez, J.A. Molina-Guillamón, A. Morales

S. de Hematologia. H. V. de la Arrixaca, MURCIA, Spain

Peripheral blood progenitors cells (PBPCs) for autologous transplant require ex-vivo manipulation in several steps. Bacterial contamination is a well known risk in most of the transplant units, although their clinical significance remains controversial. We reviewed the clinical records of our transplanted patients. The aim was to know the incidence of bacterial contamination in PBPCs, and how this affects post-transplant infections, time to engraftment, transfusion requirements, days of fever and hospitalization of our patients. A total of 134 transplanted patients received 525 aliquots of PBPCs product, a median of 3 (1-20) per patient. 127 patients (95%) had fever in the post-transplant period, 117 (92%) of them had positive blood cultures obtained from peripheral vein or central venous catheter. The most frequent bacteria were: coagulase-negative Staphylococcus in 53% patients, followed by coagulase-positive Staphylococcus in 3%, E.coli in 3%, Streptococci in 3%, and others in less than 1%. Bacterial contaminated PBPCS were infused to 11 patients. The bacteria isolated from these aliquots were: 2 Streptococcus viridans, 5 coagulase-negative Staphylococcus, 2 Corynebacterium and 2 no identified Gram-positive bacillus. These patients received no prophylactic antibiotic therapy, but at the moment of infusion peripheral blood granulocyte count were normal. In three out of these eleven patients receiving contaminated PBPCs, the same bacteria was isolated in blood. No difference was found between patients receiving grafts with and without contaminated PBPCs in terms of days of fever (3(1-7) vs 4(1-23)), transfusion requirements, days of hospitalization, days of engraftment of granulocytes (12(10-18) vs 11(9-25)) and platelets (13 (10-25)(vs 12(6-35)). In the group of patients receiving contaminated PBPCs, no difference was found between the three patients with the same bacteria and the eight with a different one it in terms of: days of hospitalization, days of fever 4(1-6) vs 3(2-7) respectively, day of granulocytes 12(10-12) vs 12(10-18) and platelets engraftment 11(10-13) vs 13(12-21) respectively. From our experience, it seems that the infusion of contaminated hematopoietic cells has not clinical translation although there are few cases. The microorganism most frequently isolated in the contaminated PBPCs aliquots and in the blood cultures of patients with fever was S. epidermidis. As this bacterium is frequently associated with vascular catheter infections, we cannot know if the contamination is due to infused product or not.

1287

THERAPEUTIC CYTAPHERESIS: AN ADAPTED STRATEGY FOR LEUKODEPLETION

P. Bergamaschi,[1] C. Perotti,[2] C. Del Fante,[2] G.L. Viarengo,[2] D. Bressan,[2] D. Sabbioni,[2] L. Bellotti,[2] V. Galiazzo,[2] L. Salvaneschi[2]

[1]*IRCCS Policlinico San Matteo, PAVIA, Italy;* [2]*IRCCS Policlinico San Matteo, PAVIA, Italy*

Background and Aims. hyperleukocytosis may represent a life-threatening condition for patients with ALL, AML, CLL or CML. Elevated white blood cells counts may cause acute respiratory distress syndrome, intracranial bleeding and the tumor lysis syndrome after chemotherapy. Therapeutic leukapheresis dramatically decreases the number of circulating leukocytes with beneficial effects on hyperviscosity and leukosta-

sis. Moreover leukocytoreduction prior to chemotherapy can reduce metabolic and renal complications due to rapid cellular lysis. Despite benefits, leukapheresis may pose multiple concerns because of patient's poor clinical conditions like concomitant anaemia, thrombocytopenia and hypertension. ASFA Committee classifies hyperleukocytosis as category I indication for therapeutic apheresis, nevertheless the effect is only temporary and the institution of appropriate chemotherapy is essential. We report the experience of performing leukocytoreduction at our Apheresis Service employing a third generation cell separator adapted on the basis of target cells separation characteristics. Methods. a summary of the patients characteristics is given in Table 1.

Table 1. Characteristics of the patients (n=16).

Male/female		10/6
Age (years)*	56	(33-85
Diagnosis:	CLL	6 (37%)
	ALL	5 (32%)
	CML	3 (19%)
	NHL with circulating blasts	1 (6%)
	AL in MDS 1	(6%)

Peripheral blood values:	PRE-apheresis	POST-apheresis
WBC (x10^3/microL)*	251.2 (87.5-514.0)	115.5 (46.6-266.0)
% of leukoreduction*	57.7 (34.2-77.9)	
Hb (g/dL)*	8.6 (4.0-11.3)	6.6 (3.7-9.6)
PLT (x10^3/microL)*	75 (6-216)	43(6-99

Table 2. Characteristics of leukapheresis (n=16).

No. of procedures per patient	2 (1-2)
Processed blood volume (liters)	9.77 (5.5-14.5)
Processed blood volume/patient blood volume ratio	9.77 (5.5-14.5)
Centrifugation speed (rpm)	1074(876-1685)
Anticoagulant infused to the patient (mL)	807 (486-1086)
Time of procedure (min)	166 (111-228)
Volume of leukapheresis (mL)	807 (486-1086)

mean value and range in brackets.

All patients signed a detailed informed consent. An immediate pre-apheresis as well as a post-apheresis blood count was carried out for every procedure; moreover, blood cell morphology was assessed by peripheral blood stream and May Grunwald-Giemsa staining. Leucocytes were removed by continuous'flow centrifugation leukapheresis (COBE Spectra® device, Lakewood, CO, USA) utilizing citrate dextrose solution A as anticoagulant. The mononuclear cells (MNC) collection program was used in all cases, switching into manual mode and appropriately modifying the separation parameters. The separation factor of the device varied from 500 to 1000 depending on the target cells size (small lymphocytes or large blasts). The collect pump rate was always set at 5.0 mL/min. Patients were carefully monitored as respect to vital signs (blood pressure, heart rate, oxygen saturation). Calcium gluconate was infused i.v. continuously to prevent or minimize citrate toxicity. Isovolemia was maintained by carefully replacing the withdrawn volume with 5% human serum albumin in 0.9% NaCl solution i.v. continuously. In case of platelet count less than $20\times10^3/\mu L$, prompt transfusion was administered before and/or after leukapheresis; red blood cell transfusion when necessary was delayed to completion of apheresis to avoid further increase in hyperviscosity. Results. from 2001 we have performed 34 apheresis procedures in 16 patients, whose characteristics are detailed in table 1 and 2. We obtained a decrease in circulating leukocytes (to less than $100\times10^3/\mu L$) by a unique leukapheresis in 7 patients (44%) and by 2 procedures in 9 (56%). No significant adverse effects occurred. Conclusions. in our hands, the strategy based on the MNC program adapted for leukocytes morphology showed to be effective as well as highly tolerated and safe. This variant provided efficient leukocytes reduction; 5% Albumin administration was able to preserve from the risks of hypotension even critically ill patients.

1288

AUTOIMMUNE HEMOLYTIC ANEMIA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

A. Kostourou,[1] S. Saridakis,[1] I. Baltadakis,[2] P. Koutsogianni[1], H. Gerovangeli,[1] A. Megalou,[1] D. Zoulas,[1] E. Kapasouri[1], M. Moschou-Parara[1]

[1]*Blood Service, Evangelismos Hospital, ATHENS, Greece;* [2]*Depart. of Hemat. & Lymph. Unit/BMT Unit, ATHENS, Greece*

The development of autoimmune hemolytic anemia (AIHA) is one of the adverse effects of allogeneic bone marrow transplantation. AIHA is thought to be due to antibodies produced by the donor's immune system against antigens on RBC's of donor origin. Measure the incidence of AIHA after allogeneic stem cell transplantation. The records of 213 patients (male:131, female:82), transplanted for hematological malignancies, referred to our immunohematology laboratory between Jan 2000 and Jan 2006, were reviewed. Patients who experienced hemolysis with a positive DAT were selected for further study. The presence of antierythrocyte autoantibody was analyzed on the red blood cells and in the serum of patients. All cases were studied using polyvalent and monospecific antisera (ID-Diamed) as well as IgG subclasses. Eluates were performed with acid elution test. A diagnosis of AIHA was made in 5 out of 213 patients (2,3%) referred to our laboratory. The median age was 24 years (range, 16-47years); 4 of them were men. Two had T- and B-ALL, one AML, one B-CLL and one aplastic anemia. Major or minor ABO incompatibility occurred in 2 cases. Three had received graft from HLA-matched related and 2 from HLA-matched unrelated donors. The onset of AIHA ranged from 4 to 13 months post-transplant (median: 7 months). The autoantibody was IgG class in all 5 patients and IgM in 2 patients. Complement (C3d) was detected on the red cell surface in all cases. In all the patients with AIHA the autoantibody was also present in the serum. The IgM autoantibodies were of broad thermal amplitude ranging from 4-30oC. The IgG subclasses were IgG1 in 3 patients and IgG3 in 1 case (4 patients evaluated). Anti-e autoantibody specificity was detected in one case. 3 patients had warm-type AIHA and 2 mixed (warm and cold) AIHA. Warm-type AIHA had an earlier onset beginning 4-7 months post-transplant, whereas mixed-type developed 12-13 months post-transplant. Selected and irradiated red cells were given to 3 patients with severe and symptomatic anemia, while the initial treatment consisted of steroids. Both patients with mixed-type AIHA are now in complete remission. One patient with warm-type has compensated hemolysis without treatment. One patient died due to resistant underlying disease and the last patient failed to respond to steroids, presented graft failure and received a second transplant. AIHA following bone marrow transplantation is a rare side effect. Its frequency ranged to 2,3% and there is a male predominance. The serologic findings are the same as in AIHA not associated with transplantation. The hemolysis may be severe and chronic. There is considerable variation in prognosis, reflecting the treatment modalities used.

1289

THERAPEUTIC PLASMA EXCHANGE FOR THROMBOTIC MICROANGIOPATHY AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION: A SINGLE CENTRE EXPERIENCE

M. Rosales,[1] A. Cunha,[2] S. Roncon,[1] L. Santos,[1] P. Pimentel[1], A. Carvalhais[1]

[1]*Instituto Portugus de Oncologia, PORTO, Portugal;* [2]*Centro Hospitalar de Vila Nova de Gaia, V N GAIA, Portugal*

Thrombotic microangiopathy (TMA) after hematopoietic stem cell transplantation (HSCT) is an uncommon but serious complication. The etiology is still unclear although endothelial damage is a common mechanism. No effective treatment is available, although the therapeutic plasma exchange (TPE) with fresh frozen plasma (FFP) could partially improve its manifestations. The aim of this study was to retrospectively analyse TMA cases in our Centre and their response to TPE. In the last 10 years, 389 patients underwent allogeneic HSCT, 10 of them (2.6%) developed TMA and initiated plasma treatment. These patients, 5 men and 5 women, with a median age of 30.5 years (range 6-49) were diagnosed with Acute Leukemia (n=4), Myelodysplastic Syndrome (n=2), Paroxysmal Nocturnal Hemoglobinuria (n=2), NonHodgkin´s Lymphoma (n=1) and Chronic Myeloproliferative Syndrome (1). It was the first allogeneic HSCT for 6 patients, the second for 3 and the third for 1 of them. The conditioning regimen was myeloablative in 5 patients and of reduced intensity in the remaining ones. The source of stem cells was peripheral blood from HLA-identical related donor for 7 patients and the other 3 received bone marrow (1 HLA- identical related, 1 mismatched related, 1 identical unrelated). Cyclosporine (CSA) was used for

graft-versus-host disease (GVHD) prophylaxis in 8 patients and Tacrolimus in the other 2. All patients had acute GVHD and 6 of them also developed chronic GVHD. Infection with cytomegalovirus was documented in 4 cases. The time of TMA onset after HSCT was 131 days (34-651). All patients had microangiopathic hemolytic anemia with 74 g/L (65-91) of hemoglobin, 4 schistocytes per field (2-10), a platelet count of 28×10^9/L (11-127), a reticulocyte percentage of 4.58 (0.18-6.55), lactate dehydrogenase (LDH) of 787.5 U/L (574-5174) and unconjugated bilirrubin of 40 µmol/L (7.7-165). These patients also presented: renal insufficiency (n=7), neurologic abnormalities (n=6), coagulation abnormalities (n=6) and fever (n=2). When TMA was diagnosed, CSA was discontinued in all patients. Plasma infusion was assigned to 1 patient, TPE to 5 and 4 began the first regime changing to TPE after 7 sessions (2-11). A total of 72 TPE, with a median of 8 (2-17) per patient, were carried out using the Cobe Spectra cell separator. Those patients received an average of 2.5 L (1.4-3.9) of FFP with a fluids change of 100% remainig isovolemics. The procedures were well tolerated although the clinical improvement was poor. After TPE, renal insufficiency was still present in the 7 patients but headache had disappeared in all of them and LDH decreased to 518 U/L (277-6268). Since the diagnosis, the overall mortality was 90% after 32 days (4-203). The causes of death were multisystem organ failure syndrome (n=7), infection (n=1) and progressive GVHD (n=1). In summary TPE has shown inconsistent results. Even the patients who responded to TPE did not have prolonged survival. However, early detection of TMA may allow advanced evaluation of the patient and change the disease prognosis.

1290

GLOBAL QUALITY OF LIFE OF PATIENTS WITH MULTIPLE MYELOMA AND MALIGNANT LYMPHOMA AFTER THE HSCT: A CROSS-SECTIONAL AND RETROSPECTIVE STUDY

L. Slovacek,[1] B. Slovackova,[2] M. Blazek,[3] L. Jebavy,[1] J. Horacek[1]

[1]*University of Defence, HRADEC KRALOVE, Czech Republic;* [2]*Psychiatric Clinic, HRADEC KRALOVE, Czech Republic;* [3]*Department of Hematology, HRADEC KRALOVE, Czech Republic*

Backgrounds. The cross-sectional and retrospective study analyses the selected factors which influence global quality of life (QoL) of patients with multiple myeloma and malignant lymphoma after the hematopoietic stem cell transplantation (HSCT). *Aims.* 1. to verify the applicability of the Czech version of an international generic European Quality of Life Questionnaire - Version EQ-5D for the evaluation of global QoL of patients after the HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic, 2. to evaluate the global QoL of patients with multiple myeloma and malignant lymphoma after the HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic, 3. to analyse factors which influence global QoL of patients with multiple myeloma and malignant lymphoma after the HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic. Patients and Methods. The total number of respondents after the transplantation from 2001 to 2003 was 80 and the return rate of questionnaires was 70% (56 respondents: 32 respondents (18 men, 14 women) with multiple myeloma and 24 respondents (11 men, 13 women) with malignant lymphoma). All respondents with multiple myeloma were after the autologous HSCT. 22 respondents with malignant lymphoma were after the autologous HSCT and 2 respondents with malignant lymphoma were after allogenous HSCT. The average age of patients with multiple myeloma was 60 years and the average age of patients with malignant lymphoma was 44,5 years. The Czech version of an international generic EuroQol Questionnaire - Version EQ-5D was used. The influence of monitored factors (age, sex, education, merital status, polymorbidity, nicotinism, religion, type of disease and the time lapse from the HSCT) on global QoL of patients was determined by means of dispersion analysis. Results. The above-mentioned factors proved statistically significant dependence of EQ-5D score and EQ-5D VAS on age (in both cases $p<0,01$), nicotinism in patients with multiple myeloma (in both cases $p<0,05$) and on type of disease (in both cases $p<0,01$). *Conclusion*. EQ-5D score and EQ-5D VAS significantly decrease with increasing age in both groups patients and with nicotinism in patients with multiple myeloma, and are significantly higher in patients with malignant lymphoma. The influence of other factors on EQ-5D score and EQ-5D VAS was not proven as statistically ignificant. The global QoL of patients with multiple myeloma after HSCT is lower (mean EQ-5D score was 68,9%, mean EQ-5D VAS was 66,6%) than in patients with malignant lymphoma after the HSCT (mean EQ-5D score was 82,7%, mean EQ-5D VAS was 76,7%).

1291

PAIN COPING MEASURING IN HEMOPHILIA- INDIVIDUAL VERSUS COMPOSITE SCORES

M. Sretenovic,[1] P. Kovacevic,[2] I. Elezovic,[3] D. Boskovic[3]

[1]*Clinical Center of Serbia, BEOGRAD, Serbia and Montenegro;* [2]*Faculty of Phylosophy, BEOGRAD, Serbia and Montenegro;* [3]*Institute of Hematology, BEOGRAD, Serbia and Montenegro*

Backgrounds. Coping with pain had been the object of studies for long time before the instruments for assessment in specific illnesses, like haemophilia, were constructed. Some research has already indicated that individual sub-scales in Cope Strategies Questionnaires (CSQ) are more meaningful than composites (*Jensen MP et al., 1992*). *Aims.* The aim of this study is to describe pain coping strategies among patients with haemophilia and find out if the results are related to the severity of disease. *Methods.* A group of 24 adult patients with moderate and severe haemophilia is presented. The patients' coping with pain is assessed using the Pain CSQ Adapted for Haemophilia (*PCSQ, Barry and Elander, 2002*). This questionnaire was translated into Serbian, according to the recquired guidelines. Clinical severity of disease is measured using the frequency of bleeding episodes in the previous year (Solovieva S, 2001). Physical activity level is measured on a two- point scale. Statistical analysis, firstly performed, was based on the three originally defined factors in the PCSQ: negative thoughts about pain, coping attempts and passive adherence. Afterwords, it was based on 14 subscales, each one grounded on of 3 to 6 items. *Results.* In the factor analysis, no differences are found in coping with pain between the groups with clinically severe and moderate disease ($p>0,05$), between patients with biologically severe and moderate haemophilia ($p>0,05$) and between those with difficulties in hard (moderate) physical activity and those with difficulties in any (no) acitivity ($p>0,05$). When using sub-scale scores, differencies in pain coping strategies were found between the groups. Patients with difficulties in hard or moderate physical activities ignored pain sensations and increased behavioural activities, using them like preferred strategies more than people with difficulties in any or no activity ($p<0,05$). On the other hand, patients with difficulties in any activity used clotting factor and ice more often to cope with pain ($p<0,05$). Patients with clinically moderate disease also ignored pain sensations more willingly than those with severe haemophilia ($p=0,026$), who relied on praying and hoping ($p=0,01$) and used anger self- statements more when in pain ($p=0,054$). Patients with biologically moderate disease used ice more than those with severe disease ($p=0,059$). *Summary*. The results based on factor analysis suggest that the severity of hemophilia may not be the factor detemining the type of patient's pain coping strategy. The results based on sub-scales analysis suggest that possibly it would be better to analyze scores from the questionnaire in this way, rather than putting sub-scales together into three factors.

1292

FATIGUE IN NEW NON-HODGKINS LYMPHOMA PATIENTS, STRATIFIED ACCORDING TO THE INTERNATINAL PROGNOSTIC INDEX

A. Novik,[1] S. Kalyadina,[2] T. Ionova,[2] A. Kishtovich,[2] D. Fedorenko,[1] T. Chagorova,[3] M. Kopp,[3] J. Lebedeva,[3] A. Myasnikov,[3] N. Malakchova,[3] N. Osipova,[2] A. Pankova,[3] G. Gorodokin[4]

[1]*National Pirogov Medical Surgical Center, MOSCOW, Russian Federation;* [2]*National Cancer Center, ST. PETERSBURG, Russian Federation;* [3]*Russian Lymphoma Study Group, MOSCOW, Russian Federation;* [4]*NJ Cent. for QoL and Health Outcome Res., NEW JERSEY, USA*

Background. Fatigue is the most distressing symptom associated with cancer. Fatigue prevalence and severity observed during prior specific treatment may help to better understand the potential underlying mechanisms and to provide patient-centered treatment. At this time, the data on fatigue severity and its prevalence in new non-Hodgkin's lymphoma (NHL) patients are lacking. The aim of this study was to describe fatigue prevalence and severity in new NHL patients, stratified according to the International Prognostic Index (IPI). *Patients and Methods*. 138 patients with newly diagnosed NHL - 76 aggressive (stage I-IV, mean age 60.0 SD=17.2, males/females 40/36) and 62 indolent (stage I-IV, mean age 59.8 SD=16.0, males/females 35/27) were included in this study. Each patient completed the Brief Fatigue Inventory (BFI) before treatment. To find out the relationship between fatigue and International Prognos-

tic Index we examined fatigue severity and fatigue interference within patient groups stratified by IPI. *Results*. Fatigue was reported by 77.3% NHL patients predominantly in those with aggressive lymphoma (93%). Almost two thirds (60.5%) of patients experienced fatigue at the moderate-to-severe level. Aggressive NHL patients reported significantly more fatigue interference with patients' daily lives than indolent NHL patients: mean BFI interference score - 5.98 (SD = 2.50) vs 2.16 (SD = 2.55) (p<0,05). The distribution of patients according to the IPI was as follows - IPI-1: IPI-2: IPI-3: IPI-4 - 5.3: 5.3: 19.3: 70.1 (%) for aggressive NHL and 40.0: 30.0: 20.9: 8.1 (%) for indolent NHL. Fatigue severity differed significantly in the IPI groups (p=0.001). Patients at low risk according to the IPI both in aggressive and indolent NHL groups had no fatigue. Patients at low-intermediate risk IPI experienced mild fatigue (mean 3.2, SD=2.3 for aggressive NHL; mean 2.1, SD=1.8 for indolent NHL). IPI-3 group was characterized by moderate fatigue (mean 5.7, SD=1.5 for aggressive NHL; mean 4.1, SD=2.4 for indolent NHL). Patients at high risk IPI had severe fatigue (mean 7.5, SD=1.4 for aggressive NHL; mean 7.1, SD=0.9 for indolent NHL). Significant differences in fatigue interference with patients' daily lives were found across IPI groups (p=0.001). *Conclusion*. Our results show that fatigue is a prevalent and distressful symptom in new NHL patients. It is much more pronounced in patients with aggressive lymphoma. Furthermore, we found that a certain IPI group is strongly distinguished by fatigue severity and its impact on quality of life. The findings support the suggestion that fatigue should be discussed as an important prognostic factor in this patient population.

1293

USAGE OF EICOSAPENTAENOIC ACID AND HIGH PROTEIN CONTAINING ENTERIC FEEDING PRODUCT IN MALIGNANCY RELATED WEIGHT LOSS OF CHILDREN

F. Erbey,[1] I. Bayram,[1] Z. Can,[2] A. Tanyeli[1]

[1]*Cukurova University, ADANA, Turkey;* [2]*Abbott Nutrition Service, ADANA, Turkey*

The aims of nutrition therapy are preventing weight loss and improving functional capacity and life quality in cancer patients. Clinic effectiveness of standard nutritional support is limited in patients with tumor related weight loss. We aimed to observe weight changes of actively chemotherapy receiving patients by using eicosapentaenoic acid and high protein containing enteral nutrition product (ProSure®) 46 patients [27 (58.7%) male, 19 (41.3%) female] were included to study. Mean age of patients was 80.5±41.38 months (20-187 months). 39 patients had diagnosis of leukemia, 7 had that of a solid tumor. All patients were receiving chemotherapy actively. Basal weight and body sizes of patients were recorded and they were suggested to use enteral products twice a day (morning - evening) in addition to their normal feeding. Patients were followed with regular intervals and their data were recorded. Their tolerance and regular use of the product were questioned. Patients were followed approximately 92±40.8 days. 33 (71.7%) patients had consumed and tolerated the product, 13 (28.3%) patients had consumed less than suggested amount because of its taste. Body weights of 20 (43.5%) patients were increased while that of 9 (19.6%) were decreased. No significant weight changes were observed in 17 (37%) patients. In conclusion, body weights of 80.4% of our patients were preserved, and that of 43.5% were increased.

1294

SATISFACTION WITH IRON CHELATION THERAPY IN PATIENTS WITH THALASSEMIA, SICKLE CELL DISEASE, AND MYELODYSPLASTIC SYNDROMES

D. Rofail,[1] M. Viala,[2] E. Trudeau,[2] L. Abetz,[1] J.F. Baladi,[3] K.A. Payne[4]

[1]*Mapi Values Ltd, BOLLINGTON, United Kingdom;* [2]*Mapi Values France, LYON, France;* [3]*Novartis Pharmaceuticals Corporation, NEW JERSEY, USA;* [4]*Caro Research Institute, QUEBEC, Canada*

Backgrounds. Thalassemia, sickle cell disease (SCD), and myelodysplastic syndrome (MDS) patients require regular blood transfusions as part of their treatment programme. Regular blood transfusions can lead to iron overload (IO). Untreated, IO will result in morbidity and early mortality. Removal of excess iron with currently available iron chelation therapy (ICT) requires 8-12 hour infusions, repeated 5-7 days per week. This time consuming therapy is burdensome to patients and may impact on satisfaction with ICT. *Aims.* To assess satisfaction with ICT and factors associated with satisfaction in patients with thalassemia, SCD, or MDS. *Methods.* A 28 item satisfaction questionnaire was developed and is currently being validated. The instrument comprises four domains that assessed satisfaction with ICT therapy (acceptability; burden; perceived effectiveness; and side effects) and was administered at a single time point to 110 patients currently receiving ICT in the US (thalassemia: n=41, SCD: n=9) or UK (thalassemia: n=40; SCD: n=14 MDS: n=6). Simple regression analyses were then conducted to explore factors explaining satisfaction with ICT. *Results.* The mean age was 30.87 years (SD=14.95), with 63 females and 47 males. A total of 54% responded that they found their ICT *inconvenient* or *very inconvenient* compared to 26% who stated that they found their therapy *convenient* or *very convenient*. Further, 28% reported they were either *very satisfied* or *satisfied* with their prior ICT compared to 31% of patients stated that they were either *dissatisfied* or *very dissatisfied*. When asked 'overall, how did the side effects of chelation therapy meet your expectations, 22% stated that they were either *much better* or *somewhat better* than their expectations, with 32% stating that they were either *somewhat worse* or 'much worse' than their expectations. Simple regression analyses revealed that whether patients experience side effects (R^2=15%, p<0.0001), and the number of doses per week (R^2=7%, p=0.007) were positively related to acceptability of ICT, whereas the number of doses missed in the last 7 days was negatively linked (R^2=6%, p=0.01). Whether patients experienced any side effects (R^2=15%, p<0.0001), patient's disease (13%, p=0.0008), age (R^2=10%, p=0.0008), and whether patients were unemployed (R^2=7%, p=0.007), were significantly associated to satisfaction with burden of ICT. Whether side effects were experienced was also significantly and negatively associated with satisfaction with side effects (R^2=21%, p<0.0001). Disease type (R^2=9%, p=0.007) and unemployment status (R^2=6%, p=0.009) were also significantly associated to satisfaction with side effects. Further, patients disease and patient's place of residence explained 8% of the variance of perceived effectiveness (p=0.02, p=0.01, respectively). *Summary/Conclusions.* Results indicated that the majority of patients found their ICT inconvenient and one third of patients stated that they were dissatisfied with their ICT. Further, satisfaction is significantly influenced by a number of important factors related directly to ICT. Whether patients experience any side effects appears to be the single most important determinant of satisfaction and was associated with three of the four satisfaction domains: acceptability of ICT; burden of ICT and satisfaction with side effects.

1295

DOWN-REGULATION OF OSTEOCALCIN BY IMATINIB-INDUCED INHIBITION OF CELL PROLIFERATION

P. Wihlidal,[1] H. Karlic,[2] F. Varga,[3] T. Nösslinger,[2] E. Pittermann,[2] M. Pfeilstöcker[2]

[1]*Ludwig-Boltzmann Institute, VIENNA, Austria;* [2]*Ludwig-Boltzmann Institute for Leukaemia, VIENNA, Austria;* [3]*Ludwig Boltzmann Institute for Osteology, Austria*

Backgrounds. Activated stemm cells from haematological malignant tomours may show also characteristics of mesenchymal stem cells. In c-kit (CD117) positive cells, at least two splice variants of osteocalcin (OCN) were described. *Aims.* Aim of our study was to elucidate whether the expression of OCN and its splice variants relate to differentiation stages of of haematopoietic cells (HL60) and an associated regulation of the transcribtion factors AML1 and AML3. Additionally, we wanted to clarify how cell-differentiating agents like vitamin D3 and imatinib (Glivec) affect proliferation of cells and expression of the genes named above. *Methods.* After incubation with differentiating agents (vitamin D3, imatinib), mRNA-expression of OCN, the transcription factors AML1 and AML3 and various metabolic genes were quantified by means of RT-PCR. *Results.* Our RT-PCR quantifications showed that after addition of vitamin D3 and imatinib, OCN appeared to be down-regulated. Alike observed at all marker genes for metabolism and haematopoiesis, the effect of inhibition of AML1 and AML3 was strongest with vitamin D3. After imatinib treatmant, in all cell-lined analysed, the dose-dependent repression of proliferation is coupled with inhibition of OCN- and AML1-mRNA-expression. As opposed to the down-regulation of markers for immature cells, the differentiation marker Lox (lysyloxidase) was stimulated. *Conclusion*: In the cell-lines observed, differentiation leads to a decrease of the expression of OCN and the associated transcription factors. Further studies shall prove the effect of differentiation agents on healthy cells.

1296

CRYOPRESERVATION OF PERIPHERAL BLOOD PROGENITORS FOR AUTOLOGOUS TRANSPLANTATION IN HEMATOLOGICAL MALIGNANCIES WITH DIFFERENT CONCENTRATION OF CRYOPROTECTANT - FIVE YEAR CENTER EXPERIENCE

A. Pivkova, L. Cevreska, Z. Stojanoski, N. Siljanoski,
A Stojanovic, S. Genadieva-Stavrik, I. Panovska,
S. Krstevska-Balkanov, O. Karanfilski, S. Trajkova, B. Georgievsk

Department of hematology,Clinical Center, SKOPJE, Macedonia

In this study we present our five year center experience with cryopreservation of PBSC and autologous transplantation in 42 patients with hematological malignancies treated in a period 2000-2005 at Department of Hematology, Skopje. *Material and Methods.* diagnosis of patients were (9 AML, 11 NHL, 10 MM, 12 HD) and median age at transplant was 34 years (7-63). Mobilization of PBSC was provided with Etoposide (VP-16) + G-CSF 10mcg/kg in AML patients, and high dose Cyclophosphamide 4-5gr/msq+G-CSF 10 mcg/kg or alone G-CSF 10 mcg/kg in patients with limphoproliferative diseases. Collected PBSC were cryopreserved in solutions with 5% DMSO in 20 patients and 10% DMSO in 22 patients, computer programmed until -80°C, and stored different period in liquide nitrogen on -196C. Autologous transplant was preformed with conditioning consisted of myeloablative high-dose chemotherapy, BuCy in AML patients, high dose Mel in MM patients, BEAM or hd ICE in NHL patients and BEAM in HD patients. Cell viability was assessed by fluorescence microscopy using acridine orange dye exclusion. *Results.* A total of 103 PBSC cryopreservation procedures were preformed in our group of patients with median 3 (2-5) apheresis procedures. Median period from storage of cryopreserved PBSC grafts until thawing was 46 days (32-60). Total number of infused $CD34^+$cells was between 2,0-15×10^6/kg and median number of mononuclear cells was 4,2×10^8/kg(1,7-7,2). The amount of infused DMSO solution ranged between 210-650ml (median 430 mL) with DMSO concentration ranging 23 ml- 50 mL (median 35 mL) in a group preserved with 10%DMSO and 13-23 ml (median 19 ml) in 5% DMSO cryopreserved grafts. The viability of the fresh harvests before storage vas median 97% (range 68, 5-99, 9%). The poorer viability was associated with harvest cell count. Bellow 300×10^9/L the median viability was 98% and only 2/42 cases had <85% viable cells. Harvests count above 300×10^9/L the median viability was 78% (67,8%-99%). In a group of patients that received PBSC grafts preserved with 10% DMSO, also revealed signs of mild DMSO infusion related toxicity (22%vs14%). Hematopoetic recovery was similar in both groups, for Ne>0,5×10^9/L on day +9 (8-10), Plt >20×10^9/L on day +12 (11-14). Our results confirm that the infusion of cryopreserved autologous PBSC in hematological malignancies revealed successful engraftment in all patients and good cell viability. We did not registered *hard to mobilize* patients and graft failure.

1297

NO SIGNIFICANT INCREASE OF CIRCULATING $CD34^+$ STEM CELLS IN PATIENTS AFTER ISCHEMIC STROKE

B. Höchsmann,[1] P. Schauwecker,[2] C. Fischer,[2] A. Storch,[3]
H. Schrezenmeier,[2] R. Huber,[3] M. Wiesneth[2]

[1]*University of Ulm, ULM, Germany;* [2]*Transfusion Medicine, University of Ulm, ULM, Germany;* [3]*Department of Neurology, University Ulm, ULM, Germany*

Backgrounds. Stroke has a great socio-economic impact. Despite this the therapeutic influence on outcome is limited. There is an increasing suggestion that hematopoetic stem cells (HPC) may be able to induce repair processes after an ischemic event. *Aim*: Our objective was to determine whether patients with an ischemic cerebral event have elevated CD34+ HPC in peripheral blood as a marker for induction of the postulated autologous repair processes. *Methods.* Total leukocyte and CD34+ cell count in peripheral blood were measured in 28 patients after MRI-verified ischemic stroke at time of admission and compared to the results of a control group of 10 healthy blood donors. Furthermore time course of these parameters was analyzed 12 hrs, 1 day, 3 days and 7 days after admission. CD34+ cell counts were assessed with FACSort according ISHAGE protocol using a dual platform method. The total leukocyte and granulocyte count was evaluated by a Coulter MDII analyzer. *Results.* The 10 healthy blood donors had a mean leukocyte count of 6.1 ± 1.4 G/l with 0.033 ± 0.028% CD34+ cells and a CD34+ cell count of 1.85 ± 1.45/µl. Mean age of the 28 stroke patients (10 females/18 males) was 67,7±12 years. 26 of the patients had an ischemia of the MCA, 2 of the ACA, 1 of the PCA and 1 of the PICA. They showed a mean leukocyte count of 6.9±3.3 G/l, with 0.027±0.016% CD34+ cells and 1.66 ± 1.05/µl CD34+ cells at time of admission. Measurement showed a mean leukocyte count of 8.1 ± 3.7 G/l, with 0.018 ± 0.022% CD34+ cells and 1.51±1.08/µL CD34+ cells 24 hours after admission. 3 days after admission measurement showed a mean leukocyte count of 7.3±3.1 G/l, with 0.021±0.022% CD34+ cells and 1.33±0.76/µl CD34+ cells and 7 days after admission a mean leukocyte count of 7.1±1.8 G/l, with 0.027±0.030% CD34+ cells and 2.00±1.10/µl CD34+ cells. There was no significant difference in leukocyte count or circulating CD34+ cell count between stroke patients and the healthy control group. 24 hrs after admission patient leukocyte counts peaked potentially explained by an acute phase reaction. However absolute $CD34^+$ cell counts of the complete patient group and of so far analysed subgroups of cortical, subcortical and territorial stroke remained without a statistically significant change. But subgroup analysis between cortical, subcortical and territorial stroke patients as well as time from stroke to admission seems to show a trend for correlation with $CD34^+$ cell number, leucocyte counts and granulocyte counts. *Summary/Conclusions.* We have found no evidence of a general increase of circulating $CD34^+$ stem cells in stroke patients that would indicate an involvement in a postulated repair process. But a subgroup analysis of a larger patient group is necessary to elucidate a possible association between circulating $CD34^+$ cells and stroke. However, the possibility that $CD34^+$ cells home to the site of tissue damage without a measurable increase of circulating $CD34^+$ cells still remains.

1298

ENHANCED ENGRAFTMENT OF HUMAN UMBILICAL CORD BLOOD DERIVED CD34+ STEM CELLS IN BALB/C MICE BY COTRANSPLANTATION OF MESENCHYMAL STEM CELLS

B. Delalat, A. Pourfathollah, H. Mozdarani, M. Soleimani,
A. Movassaghpour, S. Kaviani, A. Atashi, S. Rasi Ghaemi

Tarbiat Modarres University, TEHRAN, Iran

Backgrounds. Umbilical cord blood (UCB) is considered as an attractive alternative source of hematopoietic stem cells for allogeneic stem cell transplantations. However the rate of UCB $CD34^+$ stem cells graft is low. Mesenchymal stem cells (MSC) have been implicated in playing an important role in hematopoietic stem cell engraftment. *Aims.* In this study we examined the effect of human MSC on engraftment of human umbilical cord blood (UCB)-derived $CD34^+$ cells in irradiated Balb/C mice. *Methods.* Human UCB $CD34^+$ cells were obtained from full-term normal deliveries. Isolated $CD34^+$ cells were counted and then cultured in Stemline II Hematopoietic stem cell expansion medium supplemented with 100 ng/mL SCF, and 100ng/ml TPO in 24-well plates. The cultures were incubated at 37°C in a fully humidified atmosphere with 5% CO2, and maintained over two weeks and half the medium was exchanged twice a week. Viability test was performed by trypan blue staining (100%). The direct determination of the absolute count of $CD34^+$ was assayed by Flow cytometry (90%). Irradiated (7 Gy) Balb/C mice (n=120) were transplanted intravenously with 0.2 to 1.0 ×10^6 UCB $CD34^+$ cells in the presence or absence of 0.25 to 1×10^6 culture-expanded human bone marrow-derived MSC. The mice in every group on day 11 after transplantation were killed and their spleen dissected. In every group colony assay were performed. For approving the presence of stem cells in colony, UCB $CD34^+$ cells labeled with super paramagnetic ion oxide (SPIO) were transplanted. After establishing the presence of colonies in spleen, Prussian blue staining was performed. *Results.* Cotransplantation of low doses of UCB $CD34^+$ cells (0.2 and 0.3×10^6) and MSC (0.5 and 1×10^6) resulted in a four-fold to five-fold increase in colony forming unit spleen, in comparison with engraftment of UCB CD34+ stem cells without MSC after 11 days. After Prussian blue staining Fe+2 granules were observed. This indicates these cells in the colony were UCB CD34+ stem cells that were engrafted. *Conclusions.* The results showed that cotransplantation of MSC with UCB $CD34^+$ cells; promote engraftment of UCB $CD34^+$ cells.

1299

EARLY HEMOPOIETIC RECOVERY AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM CELL (PBSC) TRANSPLANTATION IN RELATION TO THE NATURE OF THE INFUSED PRODUCT: UNSELECTED VERSUS SELECTED PBSC

M.J. Rodríguez Salazar, B.J. González González, J.M. Raya, R.F. Rodríguez Sánchez, M.T. Hernández García, G. González Brito, J.A. Rodríguez Lpez, F. Hernández Méndez, M.L. Brito Barroso, L. Hernández Nieto

Hospital Universitario de Canarias, LA LAGUNA - SANTA CRUZ DE TENERIFE, Spain

In the setting of autologous stem cell transplantation, purified CD34+ cell selection by immunomagnetic beads remove tumor cells from PBSC apheresis product, diminishing the relapse rate and outcome. However, some authors have reported that this *purging* may delay neutrophil and platelet count recoveries after transplantation. To analyze differences in early hemopoietic recovery (day in reaching neutrophil count > 0.5×10^9/ L and platelet count > 20×10^9/ L) after autologous PBSC transplantation, when selected versus unselected products are compared. We studied 160 consecutive autologous PBSC transplantations (79 with unselected and 81 with selected PBSC), which were performed in our center over the last ten years. There were not statistically significant differences between both groups of patients in terms of infused cellularity: Unselected PBSC 3.86×10^6/kg and selected PBSC 3.33×10^6/kg (p=0.306). All patients received G-CSF daily (300 µg, subcutaneous) from day +7 until Neutrophils count > 500/ mm^3, for 3 consecutives days or Neutrophils count > 1000/mm^3. We did not find differences between the two groups in the day in which neutrophil early graft took place [unselected PBSC product, day +11,11; selected PBSC product, day +11,31 (p=0,104)]. Similarly, platelet recovery were not significantly different [unselected PBSC product, day +12,77; selected PBSC product, day +13.09 (p=0.101)]. When analysis was performed based upon the infused cellularity, we found the following Results. 1) CD34+ cells infused < 2 x 10^6/kg: unselected PBSC product, day +12,17 for neutrophils recovery, vs day +12,50 for selected PBSC product (p = 0,747) and unselected PBSC product, day +18 for platelets recovery vs day +19,83 for selected PBSC product (p=0,857). 2) CD34+ cells infused 2- 4×10^6/kg: unselected PBSC product, day +11,12 for neutrophils recovery, vs day +11,57 for selected PBSC product (p=0,210) and unselected PBSC product, day +12,46 for platelets recovery vs day +13,11 for selected PBSC product (p=0,159). 3) CD34+ cells infused > 4×10^6/kg: unselected PBSC product, day +10,40 for neutrophils recovery, vs day +10.83 for selected PBSC product (p=0,757) and unselected PBSC product, day +11,87 for platelets recovery vs day +12,43 for selected PBSC product (p=0,287). In our study, we demonstrate that, although there is a tendency to a more delayed early graft for selected PBSC products, there were not statistically significant differences between selected and unselected autologous PBSC transplantations in terms of early hemopoietic recovery. This setting did not substantially vary when infused cellularity in each group was compared.

1300

EVALUATION OF MESENCHYMAL STEM CELL EFFECT ON HOMING OF UMBILICAL CORD BLOOD STEM CELL IN BALB/C MOUSE WITH CLINICAL 1.5-T MRI.

A.A. MovassaghPour,[1] H. Mozdarani,[1] S. Akhlaghpoor,[2] A.A. Pourfathollah,[1] M. Soleimani,[1] S. Kaviani,[1] B. Delalat[1]

[1]*Tarbiat modares University, TEHRAN, Iran;* [2]*Novin medical radiation Institute, TEHRAN, Iran*

Backgrounds. MSCs have been implicated as playing an important role in hematopoietic stem cell engraftment in co-transplantation experiments. We evaluated the effect of these cells in the homing of umbilical cord blood isolated CD34+ cells in irradiated and cyclosporine received mice by using clinical 1.5-T MRI. *Aims.* Making use of clinical 1.5-T MRI for tracking transplanted hUCB CD34+ cells in animal models and to evaluate the effect of MSCs in homing of these cells. *Methods.* Culture-expanded bone marrow derived MSCs were characterized by immune phenotyping and cultured under conditions promoting differentiation to osteoblasts or adipocytes. Culture-expanded umbilical cord blood-derived CD34+ cells were labeled with iron-oxide nanoparticles (Endorem™). Irradiated (7.5 Gy) and cyclosporine received Balb/c mice were transplanted intravenously with labeled UCB CD34+ cells in the presence or absence of culture-expanded MSCs. Mice underwent MR imaging with 1.5-T MRI equipment, before and after intravenous injection of hUCB CD34+ cells labeled with SPIO through simple incubation with protamin sulfate. *Results.* After injection of iron oxide-labeled hematopoietic cells, a significant decrease in MR signal intensity was observed in the bone marrow. The signal intensity reduction in bone marrow was significantly stronger after co-transplantation with MSCs, compared to transplantation of UCB CD34+ cells alone. Histochemical examination for Iron by the Prussian Blue Method in spleen colony forming units, confirmed these results. *Conclusion*: Co-transplantation of hMSCs with UCB CD34+ cells enhances their engraftment. This can be detected and evaluated *in vivo* with clinical 1.5-T MRI.

1301

HEPATIC VENO-OCCLUSIVE DISEASE : A SINGLE CENTER EXPERIENCE WITH DEFIBROTIDE

G.T. Sucak, S.Z. Aki, M. Yagci, Z.A. Yegin, R. Haznedar

Gazi University Faculty of Medicine, ANKARA, Turkey

Backgrounds. Veno-occlusive disease (VOD) of the liver, renamed as sinusoidal obstruction syndrome is a common and possibly fatal complication of hematopoietic stem cell transplantation. The incidence of hepatic VOD being 10 - 60% varies according to diagnostic criteria and conditioning regimens. We retrospectively evaluated the laboratory values and daily progress forms of 65 consecutive cases who underwent hematopoietic stem cell transplantation. *Patients and Methods.* Sixtyfive consecutive patients with various hematologic malignancies underwent HSCT and 68 trasplantations were performed (31 autologous SCT, 37 allogeneic SCT with 3 of them being non-myeloablative SCT) between September 2003 to February 2006 with a median age of 42,5 years (range 16 - 71 years). Three patients received 2nd transplants, one as a part of tandem auto/allogeneik protocole and two of them as retransplants after relaps of their disease. As a VOD prophylaxis, all patients received ursodexycolic acid, N-acetylcysteine and continous infusion of low dose heparin. The conditioning regimens consisted of cyclophosphamide/TBI, cyclophosphamide/busulfan and cyclophosphamide/busulfan/ fludarabine for the patients reported as VOD. VOD was clinically diagnosed with the development of two of the following features: hyperbilirubinemia > 2 mg/dL, hepatomegaly with right upper quadrant pain, and ascite or unexplained weight gain (> 5% increase of baseline body weight) within 30 days of transplantation. Patients were said to have multiorgan dysfunction if there was documentation of dysfunction of one other system in addition to liver. Eleven patients (16,2%) who were diagnosed as VOD were treated with defibrotide intravenously in doses ranging from 10 to 20 mg/kg per day for a median of 10 days (range 4 - 25 days) . Serious adverse events due to defibrotide was not seen. At diagnosis median bilirubin was 4,6 mg/dL, median weight gain was 8.6%, ascite was present in 45,5% and hepatomegaly ± right upper quadrant pain was present in 81,8% of patients. Severe VOD associated with multiorgan dysfunction was present in 2/11 patients (18,2%) with a 100% mortality rate before day 100. Severe VOD was reported to have a mortality rate approaching 100% by day +100 after transplantation which we also experienced in our 2 patients. In general 16/65 patients died (24.6%) and 6/65 (9.2%) of these deaths happened before day +100. Two out of six deaths (33%) happened before day +100 were due to VOD. Complete resolution of VOD was seen in 81,8% with a survival rate of 54.5% at day +100. *Conclusion*: Although there is still no satisfactory recommendation for the treatment of severe VOD, defibrotide seems to be the best therapy reported with acceptible side effects. In generally complete resolution of VOD was reported as 36 - 42% in the literature. The favorable complete response rate which we achieved in our series may be due to the early intervention of defibrotide therapy with the diagnosis of moderate to severe VOD in addition to the prophylaxis with ursodexycolic acid, N-acetylcysteine and continous infusion of low dose heparin.

1302

CIRCULATING CFU-GM DURING HEMATOPOIETIC RECOVERY AFTER PERIPHERAL BLOOD TRANSPLANTATION: RELATIONSHIP TO GRANULOCYTE ENGRAFTMENT.

M. Albo, J. De la Fuente, C. Ares, E. Feteira, M. Alonso

Complexo Hospitalario de Vigo, VIGO, Spain

Hematopoietic progenitor cells (HPC) are circulating in the peripheral blood (PB) before engraftment following auto or allo-PBSCT. It is kwon that CFU-GM constitutes a part of primitive progenitor cells. In the present study we investigated the kinetics of CFU-GM circulating in PB during the early stages following PBSCT. *Patients:* Forty seven auto and nine allo-PBSCT recipients were consecutively selected from January 2000 to August 2002 for this study. The median age was 46.5 years (17-69). 20 patients had non-Hodgkin's lymphoma, 16 myeloma, 8 Hodgkin lymphoma, 7 acute leukemia, 4 solid neoplasms and 1 patient chronic

myeloid leukemia. Cell preparation: 10 ml of PB in heparin was obtained on days 1, 4, 9, 11, 14, 16 y 18 after PBSCT. Mononuclear cells were isolated by Ficoll-Hypaque method. Progenitor assay: A standard methylcellulose colony assay (MethoCultTM GF H4531, StemCell Technologies, Vancouver, Canada) was used for analysing the number of CFU-GM on all of the days. Statistical analysis: Routines within SPSS (Statistical Package for the Social Sciences) were used for the estimates shown. CFU-GM decreased to undetectable on day 4 after transplantation. They reappeared from day 9 to day 18 after transplantation, depending on the patient, along with neutrophil recovery. Figure 1 shown the post transplant CFU-GM kinetics. We report on the detection of GFU-GM in 13 of 56 patients on day 9 and they number ranged from 2 to 10 per 10 ml PB depending on individual patients. On day 11 we detected CFU-GM in 43 patients, the number of them was 5-12. The number of CFU-GM on day 14 was 5-13 and they were detectable in 54 patients. On day 16 and 18 almost every patients showed CFU-GM colonies -55-, the number ranged from 6 to 14 on day 16 and 5 to 18 on day 18. The presence of PB CFU-GM correlates with time of granulocyte recovery ($p<0.005$). The numbers of CFU-GM PB were similar in the auto and allo-PBSCT. Subsets of CFU-GM were detected during the early posttransplant period, they have exponential increase between days 9 and 14 and then they have few changes on days 14 to 18. CFU-GM colonies correlated with granulocyte recovery.

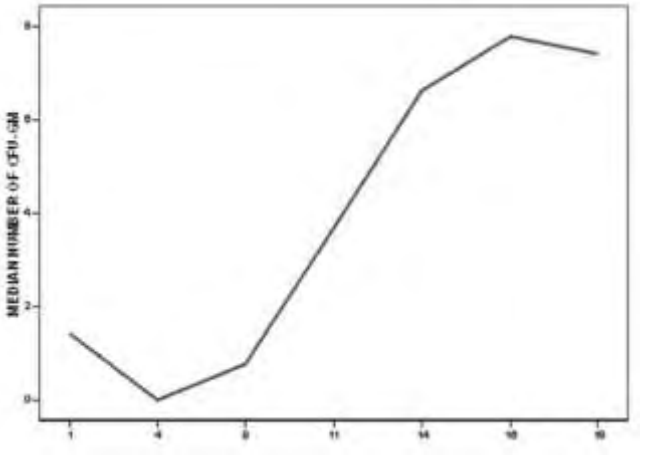


Figure 1. Kinetics of CFU-GM in PB after PBSCT.

1303

SIDE EFFECT OF STEM CELL MOBILIZATION WITH GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF) ON MORPHOLOGY AND FUNCTION OF LIVER IN MICE

V. Todria, I.B. Kaplanskaya

National Hematology Research Centre, MOSCOW, Russian Federation

Mobilization of hematopoietic stem and progenitor cells (HSPC) from the bone marrow into circulation can be induced in patients and animal models by a wide variety of molecules including hematopoietic cytokines, such as recombinant human granulocyte colony-stimulating factor (rhG-CSF). A cytokine-mobilized HSPC may 'home' to peripheral hematopoietic organs as well as to other sites, for example to liver. However, the morpho-physiology of liver during this process and the safety of G-CSF mobilization are poorly appreciated. To address this problem, the morphology and function of liver in healthy mice after short treatment with G-CSF were investigated. Female (CBAx C57Bl/6)F1 mice 16-20 week-old of age were used. The mobilization of HPSC was induced with 200 mg/kg of rhG-CSF injected daily for 8 days. The mice were killed 1, 7 and 28 days after last injection of G-CSF. Peripheral blood was collected and white blood cells were counted. Cell morphology was evaluated on May-Grunwald-Gimsa-stained blood smears. The liver function was monitored by serum bilirubin, enzyme alanin- and aspartat aminotransferase (ALT and AST) levels. Formalin-fixed, paraffin-embedded liver sections were stained with hematoxylin-eosin and Picro-Sirius red for histological examination of livers. Beginning at day 1 after last injection of G-CSF till the end of experiment (day 28) the number of peripheral blood leukocytes did not change and was the same as in control mice. Differential analyses performed on blood smears revealed that the number of mature neutrophils increased significantly after treatment of mice with G-CSF, reaching maximal values on the day 7 after abolition of cytokine. Liver in mice receiving G-CSF revealed classic liver lobules with normal architecture and with only mild hepatocellular necrosis. However, on day 7 after the last injection of G-CSF numerous erythrocytes were observed in the lumina of the central and lobular veins. Some erythrocytes and hemosiderin-containing macrophages and the foci of granulopoiesis were visible outside the vessels mainly around the portal area. Destruction of erythrocytes accompanied with slight bilirubin and AST elevations. At day 28 after mobilization most of the vessels were empty but liver sinusoids were distinctly dilated. Unexpectedly, it was observed that almost all blood vessels walls were reinforced and symptoms of fibrosis were visible. Liver vessels walls composed of a single layer of endothelial cells and no mitosis among them were visible. Picro-Sirius red positive staining revealed the presence of collagen synthesis since day 7 (all vessels) till day 28 (mainly portal tract vessels) after mobilization. Distribution and density/friability of the collagen fibers network was more prominent at the end of experiment. A strong correlation between liver morphology and microsomal enzyme induction was not demonstrated. G-CSF treatment causes morphological, but no functional changes in murine liver. The side effects observed were associated with extramedullary granulopoiesis in liver and with liver blood vessels thrombosis. These adverse effects were partly reversible at 4 weeks post-mobilization. G-CSF stimulates indirectly liver stromal cells to produce collagen, however, time-dependent collagen degradation was not observed in this set of experiment. A long-term follow up is required.

1304

THE EFFECTIVENESS OF PREOPERATIVE ERYTHROPOIETIN IN PEDIATRIC SURGERY

S. Glamocanin,[1] R. Kacarska,[1] P. Petrovski,[2] P.E. Lange,[3] O. Muratovska,[1] K. Martinova,[1] Z. Antevska,[1] B. Conevska[1], S. Koceva[1]

[1]*University Children's Hospital, SKOPJE, Macedonia;* [2]*University Pediatric Surgery, SKOPJE, Macedonia;* [3]*Deutsches Herzzentrum Berlin, Angeborene, BERLIN, Germany*

Backgrounds. The idea about bloodless surgery because of patients' personal or religious concerns, especially in the pediatric population, is challenging. Concerns about the transmission of the human immunodeficiency virus (HIV) have driven the evolution of surgical transfusion practices including the use of preoperative erythropoietin (EPO). Despite the fact that the consequences of transfusion-related diseases are very important issue for pediatric population only a few studies have examined the potential benefits of preoperative erythropoietin in children. *Aims.* The aim of this study is to assess the clinical efficiency of EPO as preoperative treatment in two children scheduled for heart and abdominal surgery without blood transfusion. *Methods and Results.* Two children are presented in this study: 15 and 3 year-old boys Jehovah's Witness. They underwent a major surgical procedure without blood transfusion (surgery of the choledochal cyst and corrective treatment of Fallot's tetralogy). Patient that is 15 years old suffered an abdominal pain and presented right upper abdominal mass in association with jaundice eight months before. He underwent clinical, laboratories examinations and ultrasonography. The diagnosis of the choledochal cyst was delivered. Surgery was made at the Department of Pediatric surgery, Medical Faculty in Skopje. The second patient, 3 years old, had discrete acrocyanosis and fatigue. Diagnosis of Fallot's tetralogy was established by clinical, laboratory examinations, ECG and Echocardiography. A successful surgery procedure was performed at the Pediatric cardiology, Deutsches Herzzentrum- Berlin. Both operations were carried out without the use of blood products through the application of multidisciplinary effort. Preoperative EPO treatment was administered subcutaneously with 300 U/kg weekly for four weeks (three weeks before surgery and one after surgery). In addition, patients received an oral iron, folic acid and vitamin C supplementation. The effectiveness of preoperative EPO treatment was followed through the RBC count, hemoglobin concentration, hematocrit, and reticulocyte count. Stimulation of erythropoiesis was seen with an increase of the reticulocyte count (42 and 61‰) by day 3 of the treatment. The increase of the hematocrit (first patient: from 39.2 to 44%; second patient: from 40.1 to 43.6%), hemoglobin concentration (first patient: from 13.7 to15.1: second patient: from 12.3 to 14.7g/dl) and increase of RBC count were registered after 3 weeks of treatment with EPO. Both patients didn't receive blood transfusion. EPO caused no adverse reactions. *Conclusions.* Preoperative EPO treatment has been shown to be an effective alternative to red-cell transfusion in children undergoing surgery. A significant hematopoietic response with no side

effects was achieved in this study. With respect of this study, but also in accordance with data available from literature, preoperative EPO may be used more often in pediatric surgery.

1305

EVALUATION OF IL-1β, IL-2, IL-4 AND TNF-α IN PATIENTS WITH THE MALIGNANT HEMATOLOGIC PATHOLOGY

M. Tsvyetkova,[1] T. Shlyahtichenko,[1] J. Minchenko,[1] K. Bruslova[1], V. Khomenko,[2] V. Bebeshko[1]

[1]*Research Center of Radiation Medicine, KIEV, Ukraine;* [2]*Kiev BMT Center, KIEV, Ukraine*

Interleukines (IL) are the main homeostasis regulating agents and they have very wide spectrum of different biologic effects. They take part in regulation of all components of immune system and execute local immune answer in malignant process but the question about how interleukines realizing their enormous possibilities for anti-tumoral resistance activation and why these possibilities may by diminished in organism with growing tumor mainly are unclear now. Optimization of prognostic criteria and efficiency of treatment for patients with different malignant hematological pathology on the basis of IL-1-β, IL-2, IL-4 and TNF-α monitoring. 39 patients (3-61 years) with different malignant pathology (acute leukemia -22 (recipients of autoPBSCT (AL)-8 and children ALL group-14); HD-8 ; MM - 9 were investigated. 25 pts received autoPBCST. For patients undergoing autoPBCST the investigation carried out at the following points: before conditioning treatment, after autoPBCST in time of restoration of hemopoiesis, through +3 - +17 months after transplantation. For children with ALL underwent only for conventional chemotherapy, investigation executed in the acute period and in the remission. The evaluation of spontaneous IL-1β, IL-2, IL-4, TNF-α production in supernatants of daily cultures of peripheral blood mononuclear cells were measured by ELISA (Diaclone, France). The control group consisted of 22 healthy persons. Very low IL-1β levels were revealed in all patients groups before auto PBSCT, its level fluctuated within 13-63 pg/ml limits. In the early post-transplant period IL-1β level raised up to 162,28+38,95 pg/ml only in patients with HD (p<0,05), meanwhile in AL group Il-1β level continue to come down (range 7,97-13,47 pg/mL) and in MM group was stable low. IL-2, Il-4 levels in PBSCT recipients group did not differ reliable from those in control in all points of observation except HD. There were higher IL-2, Il-4 level in these patients before transplantation (79,11+48,5 pg/ml and 3,29+1,21 vs 13,62+0,7 2 pg/mL and 1,25+0,52 pg/ml in reference group respectively, p<0,050), which became almost normal after PBSCT (20,07+3,77 pg/ml and 1,40+0,58 pg/ml , p<0,05). TNF-alfa level were low in HD before transplantation and in ALL relapse groups (61,05+ 17,74 pg/mL and 85,4+25,50 pg/ml vs reference 414,94+101,01 pg/mL). In HD early post-transplant period and in ALL remission group TNF-alfa level raised up to normal value (401,27+111,34 pg/mL and 320,28+123,04 pg/mL, respectively, p<0,05). The obtained data indicate the preliminary activation of patient's immuno-competent cells *in vivo*. The certain differences in IL-1β, IL-2, IL-4, TNF-α levels in view of disease, its course, treatment with PBSCT and outcome were revealed. The presented data reflects the implications of inflammatory cytokines (TNF-alfa, IL-1β) in the pathogenesis of AL in children and HD as treatment effectiveness with autoPBCST implement. We conclude that investigation of these cytokines production may be helpful in optimization of prognostic criteria and treatment effectiveness of the specified pathology.

1306

ERYTHROPOIETIN SIGNALING IN PANCREATIC TUMOR CELL, AR42J: ACTIVATION OF MITOGEN ACTIVATED PROTEIN KINESES AND THEIR EFFECT UPON CELL PROLIFERATION

B. Udupa, C. Bose

University of Arkansas for Meidcal Sci, LITTLE ROCK, USA

Erythropoietin (EPO) regulates the proliferation and differentiation of erythroid progenitors via its receptor, EPO-R and through various Mitogen activated protein kinase (MAPK) pathways. We reported last year at 10th EHA Congress that high dose EPO could enhance the proliferation of rat pancreatic tumor cell line, AR42J. *Aims.* To extended this study to examine the activation of two MAPKs, namely, extracellular regulated kinase 1/2 (ERK1/2) and c-jun NH2-terminal kinase 1/2 (JNK1/2) after exposure of AR42J cells to a physiologic dose of EPO, 5 mU/ml. AR42J cells were cultured, exposed to 5 mU/mL of EPO and cell extracts were prepared. These were separated by electrophoresis and subjected to Western blot analysis. EPO induced proliferation was evaluated by 5'-Bromo-2'-deoxyuridine (BrdU) incorporation method. We found a rapid activation of ERK-1/2 in AR42J cells reaching the maximum of 3.3 fold in 5 min after EPO exposure, while it took 30 min for JNK1/2 to reach the maximum. To examine the effect of induction of MAPK by EPO on AR42J cell proliferation, cells were treated with inhibitors to ERK1/2 and JNK1/2, PD98059 and SP600125, respectively, for 1hour prior to EPO addition and the cell proliferation were measured from day 1 through 4. EPO addition to AR42J cell culture resulted in significantly higher proliferation on day 2 and it was 1.93±0.09 absorption units compared to the value of 0.47±0.05 absorption units seen in controls without EPO at that interval ($p < 0.01$). When cells were treated with ERK1/2 inhibitor prior to the addition of EPO, proliferation was significantly suppressed to 0.53±0.05 absorption units at that interval ($p < 0.01$). Similarly with JNK1/2 inhibitor and EPO a significantly decreased cell proliferation (0.43±0.06 absorption units, p<0.01) was observed. These results indicate that in AR42J cells, for EPO mediated proliferation, activation of both ERK1/2 and JNK1/2 are necessary and indicates a role of MAPK in EPO induced proliferation of tumor cells. This aspect has to be taken into account for any treatment involving EPO.

1307

EXPRESSION OF LYMPOID T-MARKERS ON GRANULOCYTES AFTER GM-CSF AND G-CSF ADMINISTRATION

A. Kousoulakou,[1] E. Terpos,[2] J. Meletis[3]

[1]*Onassis Cardiac Surgery Center, ATHENS, Greece;* [22§1] *General Airforce Hospital, ATHENS, Greece;* [3]*Univercity of Athens, Laiko Gen. Hosp., ATHENS, Greece*

Backround: Granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) affects the function and phenotypic expression of granulocytes by different mechanisms that include: (a) direct activation of granulocytes; (b) indirect activation due to the production of other cytokines (such as interleukin-6, and tumor necrosis factor-α); (c) increased mobilization rate from the bone marrow, and therefore granulocytes still express immature markers in peripheral blood; and (d) G-CSF acts on granulocyte precursors and reduces the period of maturation in bone marrow. The effect of GM-CSF and G-CSF on granulocytes function and phenotype is complicated and depends on the dose, the route and the way (bolus, continuous) of growth factor administration. There are also differences between in vitro and *in vivo* effects. *Aim*: The aim of this study was to evaluate the effect of GM-CSF and G-CSF on granulocyte phenotypic expression. *Methods.* We determined the phenotypic expression of granulocytes obtained from the peripheral blood of 26 patients with hematological malignancies (7 patients had AML, 7 ALL, 4 CML, 5 non-Hodgkin's Lymphomas, and 3 MDS) and 7 patients with solid tumors. Blood samples were collected on the 1st and 2nd day of growth factor administration, as well as on days 5-20 after the final day of growth factor injection. Phenotypic analysis was performed by the alkaline phosphatase (APAAP) immunocytochemical technique, using a wide panel of monoclonal antibodies. Results. Granulocytes expressed T antigens on their surface after GM-CSF or G-CSF administration as depicted in table 1. High percentage of granulocytes expressed CD4 on their surface, in most cases after the 5th day from the beginning of GFs' administration. In addition, they expressed transiently CD7 and CD2 at the same time or later in relation to CD4 expression. *Conclusions.* CD2, CD4 and CD7 are adhesion molecules and they act as activation molecules. CD4 and CD7 are also markers of immaturity. The expression of those antigens on granulocytes indicates: (a) premature differentiation of granulocytes resulting in preservation and expression of immature markers on their surface; and (b) activation of granulocytes. The in vivo administration of GM-CSF and G-CSF induces the expression of T antigens on granulocytes during activation of the antigen-presenting system.

Table 1. Percentage of CD2+, CD4+ and CD7+ granulocytes.

AML	0-30	0-5	0
ALL	0-75	0-50	0-35
CML	0-30	0-20	0-25
MDS	0-65	0	0-5
NHL	0-80	0-35	0-45
Solid tumors	0-50	0-65	0-70

1308

DIFFUSE ERYTHEMATOUS,MACULO-PAPULAR RASH, FOLLOWING GCSF IN A LUPUS PATIENT PRESENTING WITH NEUTROPENIA, MILD THROMBOCYTOPAENIA AND HYPONATREMIA

S. Mitra, P.T. Murphy

Beaumont Hospital, DUBLIN, Ireland

Backgrounds. G-CSF(granulocyte colony stimulating factor- eg Filgrastim) regulates the production of neutrophils within the bone marrow and also impacts on their function.It is frequently given to patients with leucopaenia or neutropenia caused by various underlying diseases.The treatment with GCSF is apparently safe although flares in patients with autoimmune diseases and cutaneous vasculitis have been described in the literature. Selected patients with myelodysplasia(MDS) with suppurative infections may be a candidate for GCSF although there is no data to support their routine use in MDS. Other common side effects of G CSF are musculoskeletal pain, transient hypotension, deranged LFTs, thrombocytopaenia, dysuria, proteinuria, haematuria, allergic reactions and transient decrease in blood glucose. Splenic enlargement, hepatomegaly, headache, diarrhoea, epistaxis, alopecia, osteoporosis and reactions at injection site have been reported.

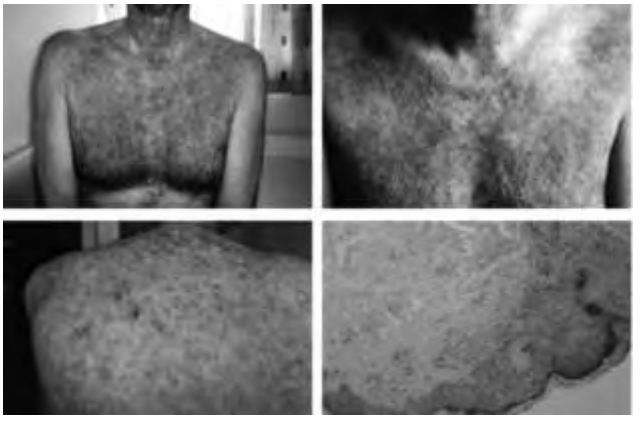


Figure 1. Rash following G-CSF/histology of rash.

Case history:We report a 55 yr old man who presented to the A/E with a 2 week history of feeling generally unwell with decreased appetite, muscular aches and pains (all joints) and nausea He had background history of Type II Diabetes Mellitus and hypercholesterolaemia. Blood tests on admission revealed that he had neutropaenia (N=0.6) mild anemia(Hb =12) mild thombocytopaenia and hyponatremia(Na 123). His bone marrow raised the possibility of early myelodysplasia although his karyotype was normal. An ultrasound scan of the abdomen revealed mild splenomegaly of 14.4 cm.A CT scan revealed a pulmonary nodule 1x2 cm. A lung neoplasia as aetiology of his hyponatremia was queried.However a PET scan was negative. He presented to the A/E two weeks later with febrile neutropaenia and was treated with intravenous broad spectrum and G CSF subcutaneously the following day. About 7 days later he developed a non itchy widespread maculopapular rash over his scalp, trunk(front and back) arms (distally) and legs. G CSF was discontinued. A skin biopsy was consistent with leucocytoclastic vasculitis.Further investigations revealed: ANA positive in 1/400 ;dsDNA positive in High titre; complement titres-low. SLE seemed likely diagnosis and he was commenced on Prednisolone(1mg/kg). His hyponatraemia settled and his rash disappeared in a week's time.His leucopaenia was slow to resolve. After 3 weeks of Prednisolone 1 mg/kg his WCC was 2.58 and Neutrophil count was 1.54. *Conclusion*: GCSF is often prescribed very freely in leucopaenia caused by multisystem disorders.In this case it caused a severe widesprad rash which was very worrisome for the patient and the family. Hence before considering GCSF for unexplained leucopaenia an autoimmune screen should be checked along with a bone marrow aspirate/biopsy. G CSF should be avoided in untreated Lupus and other vasculitides(if possible) in view of risk of flare up.

1309

CELL CYCLE STATUS OF MOBILIZED PERIPHERAL BLOOD CD34-POSITIVE CELLS IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

S.A. Sizikova,[1] I.A. Lisukov,[2] A.D. Kulagin,[1] I.V. Kruchkova,[1] N.V. Pronkina,[1] V.V. Denisova,[1] A.V. Gilevich,[1] V.S. Kozhevnikov,[1] E.R. Chernykh,[1] V.A. Kozlov[1]

[1]Institute of Clinical Immunology, NOVOSIBIRSK, Russian Federation; [2]State Medical University, NOVOSIBIRSK, Russian Federation

Backgrounds. Mobilized peripheral blood (PB) CD34-positive cells are being increasingly used as hematopoietic support following high-dose conditioning in patient with hematological malignancies. *Aim*: To evaluate the cell cycle status of PB CD34-positive cells in patients with hematological malignancies. *Methods.* The study was performed in 28 patients in the median age of 30 years (range 7 - 55), including 13 with acute leukemia (AL), 8 with non-Hodgkin's lymphoma (NHL), 4 with Hodgkin's lymphoma (HL) and 3 patients with multiple myeloma (MM). All patients received high-dose chemotherapy followed by autologous stem cell transplantation (ASCT). Autologous PB hematopoietic stem cells were mobilized with either granulocyte colony-stimulating factor (G-CSF) (n=2) or cyclophosphomide and G-CSF (n=26). High-dose chemotherapy was managed according to a standard conditioning protocol. Samples for analysis of the cell cycle state of CD34-positive cells were obtained from the first leukapheresis product (LP). The number of CD34-positive cells was determined by flow cytometry analysis. Cell cycle and apoptosis of CD34-positive cells was studied by using the 7-aminoactinomycin D (7 AAD). *Results.* Complete remission (CR) was achieved in 17 patients with the median follow-up of 14 months (range, 6-27). Eleven patients were died due to relapses of malignancies during the 12 months after ASCT. There were patients with AL of high risk group (n=3), MM (n=2) and resistant lymphomas (n=6) who were underwent to ASCT as 'salvage' therapy. No significant differences were found in hematological recovery and transplant-related complications in these patients. Median time to an absolute neutrophil count greater than 0,5x109 /l and platelet count greater than 50×10^9/L was 15 and 20 days, respectively. LP samples contained 1,92±0,54% CD34-positive cells. The majority of CD34-positive cells were in the G0/G1 phases of cell cycle (67,28%). The mean number apoptotic CD34-positive cells was 2,37%. We noted significantly greater proportion of CD34-positive cells in SM phases of cell cycle in the relapsed patients in comparison with patients who are still in CR (36.1±4.71% vs 22.1±1.53%). *Conclusion*: Further study would be required in order to clarify the relations of CD34-positive cell cycle state and risk of relapse in patients after ASCT.

1310

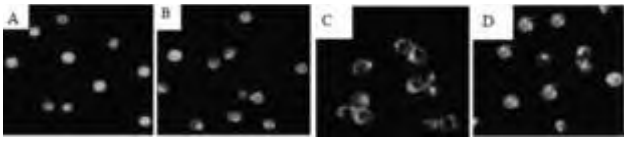
STUDY ON THE MECHANISMS OF QUERCETIN IN HL-60/ADR CELLS

F.Y. Chen, T. Wang, H.R. Wang, H.H. Huang, J.Y. Han, H. Zhong, J.H. Zhong, Z.H. Xuan, R.R. Ouyang

RenJi Hospital, SHANGHAI, China

Recently, as the constant improvement of chemotherapeutic regime, the development of bone marrow transplantation and biological target therapy, the treatment of leukemia had achieved great progress. However, a major problem in the treatment of leukemia is the development of resistance to chemotherapeutic agents. How to overcome and reverse drug resistance of leukemia cells to chemotherapeutic agents are therefore the critical issues to be solved urgently in the clinics. Recently, it has been shown that quercetin, a Chinese herb, could inhibit the growth of leukemia cells, trigger apoptosis, and even reverse the multidrug resistance of leukemia cells in vitro. But, it remains uncertain that how quercetin reverses multidrug resistance, in particuBBPPlar, the mechanisms of its impacts on membrane transporting protein, and whether it can restore the abnormal distribution of DNR in resistant cells. In the present study, we intend to investigate aforementioned effects exerted by quercetin on drug resistant cell line HL-60/ADR *in vitro*. To investigate the effects of multidrug resistance reversed by quercetin in drug resistant HL-60/ADR cell line *in vitro.* RT-PCR was employed to detect MRP1 gene expression and elucidate the impact of quercetin on its expression both in HL-60 and HL-60/ADR cells. The subcellular distributions of DNR before and after quercertin insult were measured by confocal microscopy. After being treated with different concentrations of quercertin, there were no apparent regulatory effects exerted by quercertin on MRP1 gene expression in HL-60 cells. However, quercertin could down regulate MRP1 gene expression in HL-60/ADR cells in a dose-dependent manner. In particular, at concentrations of 20 μmol/L

and 40 μmol/L quercertin respectively, there were marked down-regulation of MRP1 gene expression, as compared with mock-treated group (p<0.01). In HL-60 cell line, the DNR fluorescence was mainly distributed in the nucleus, cytoplasm and cell membrane, with nucleus intensely, cytoplasm uniformly and diffusely, membrane continuously staining pattern (Figure A). Compared with mock-treated group, the distributions of DNR fluorescence were not obviously changed after treated with different concentrations of quercertin (Figure B). However, in HL-60/ADR resistant cells, DNR fluorescence was mainly distributed in periphery region of cytoplasm and membrane, the granule was not homogeneous, and fluorescence signal was hardly seen in the nucleus (Figure C). Nevertheless, as concentration of quercertin increased, fluorescence signal was gradually increased in the nucleus and cytoplasm. When the concentration of quercertin increased up to 40 μmol/L, the fluorescence intensity almost reached level of that in sensitive cells with diffuse granule distribution(Fig. D). Altered subcellular distribution of DNR in resistant cell line was related to MDR gene formation in tumor cells. Quercetin could inhibit MRP1 function and restore the sbucellular distribution of DNR *in vitro*.



1311

RETINOIC ACID AFFECTS THE RESPONSE OF V-MYB-TRANSFORMED MONOBLASTS TO OKADAIC ACID

P. Benes,[1] V. Maceckova,[1] Z. Andrysik,[2] J. Zatloukalova,[2] J. Smarda[1]

[1]*Masaryk University, BRNO, Czech Republic;* [2]*Institute of Biophysics ASCR, BRNO, Czech Republic*

Background. Okadaic acid (OA) inhibits serine/threonine protein phosphatases 1 (PP1) and 2A (PP2A), thus inducing differentiation and/or apoptosis of various leukemic cell lines in dose-dependent manner. This suggests that PP1 and PP2A phosphatases actively participate in regulation of these processes. Moreover, retinoic acid (RA) affects expression and activity of the PP2A. *Aims*. The aim of this study was to explore the functional interactions of RA- and OA-driven pathways in v-myb-transformed monoblasts BM2. We have previously described that BM2 monoblasts ectopically expressing Jun, RA-receptor (RAR) or retinoid X receptor (RXR) proteins differentiate to macrophage-like cells upon treatment with RA while wild-type BM2 cells do not respond to RA. *Results*. In this study we found that 10 nM OA induces adherency, cell cycle arrest, phagocytic activity, production of reactive oxygen species and expression of vimentin in BM2 cells. These features that mark differentiation along monocyte/macrophage pathway are enhanced in BM2 cells upon simultaneous treatment with OA and RA. Interestingly, the 20nM OA induces rather apoptosis than differentiation of BM2 cells as documented by analysis of cell morphology, chromatin condensation, internucleosomal DNA fragmentation and fosfatidylserine translocation. This proapoptotic effect of OA in BM2 cells was inhibited by RA. *Conclusions*. These results indicate that pro-differentiation and pro-apoptotic effects of OA on BM2 monoblasts are differently regulated by RA.

Funding: This work was supported by the grant 301/03/D022 of the Grant Agency of the Czech Republic and by the grant MSM0021622415 of the Ministry of Education, Youth and Sports of the Czech Republic.

1312

STUDIES OF PNAS-2, AN ANTI-APOPTOSIS GENE

H.R. Wang, C.H. Gu, J.Y Zhu, J.Y. Han, F.Y. Chen, R.R. Ou Yan

RenJi Hospital, SHANGHAI, China

We use gene chip and find PNAS-2 (Gene ID:AF229832) is one of the down-regulated genes upon treatment of As4S4 in APL cell line-NB4, same result has been reported by other group except the configuration of arsenic sulfide is As2S3 instead of As4S4, but PNAS-2 expression unchanged in U937 and K562 leukemia cell lines. Moreover, the results of microarray are validated by RT-PCR in a time course, the expression of PNAS-2 decreased after treated with As4S4 in NB4 cells, and this effect is time dependent. We even performed similar experiment on primary leukemia cells from leukemia patient. Again PNAS-2 expression decreased significantly in APL samples after the treatment of As4S4, while no change in M4 primary cells upon the same treatment. The hypothetic protein of PNAS-2 shows high sequence similarity to a protein that is thought to be involved in apoptosis, however, no studies has characterized this gene. To learn whether *PNAS-2* has a function associate with apoptosis. pRNAT U6.1/NEO and pTracer-CMV/Bsd were used to construct both shRNA express plasmids and over-express plasmids, stable tansfected recombinant plasmids to U937 cell lines respectively. Used both antibiotics and GFP$^+$ subpopulations cell sorting through FCM to purify GFP($^+$) tansfected cells. Annexin V-APC and 7-AAD were used to stain cells, applied FCM and confocal microscopy to detect apoptosis. After antibiotic selected and GFP$^+$ subpopulations cell sorting through FCM, confocal microscopy confirmed we had got almost pure transfected U937 cells (more than 90%). The expressions of PNAS-2 in shRNA groups decreased after RNA interference had occurred, the PNAS-2 inhibition rates were 78.1% in shRNA group, 75.4% in shRNAII group and 51.4% in shRNA group. The results of apoptosis ratio from confocal microscope were: in shRNA control group was 7.3%, in shRNA I group was 14.7%, 13.8% in shRNAII group and 10.3% in shRNA III group. Mean apoptosis ratios by FCM were 3.52% in shRNA control group, 9.23% in siRNA I group, 8.85% in siRNAII group and 7.19% in shRNA III group. Paired t test showed p values were 0.0088, 0.014 and 0.1788 respectively in shRNAI, II and III group paired compared with shRNA control group. In our PNAS-2 over-express experiment, the expression of PNAS-2 in PNAS-2-pTracer recombinant plasmid transfected group increased 1.73 times than control groups. The results of apoptosis ratio by confocal microscope were: 6.90% in control-pTracer group, 3.76% in PNAS-2-pTracer group. Apoptosis ratio detected by FCM was 4.07_±0.30% in PNAS-2-pTracer group, while in control-pTracer group was 5.51%±0.12% (p=0.0096). We find inhibition of PNAS-2 by RNA interference will increase cell apoptosis both detected by confocal microscopy and FCM, and show statistical significance in shRNA I group and in shRNA II group compared with control group. While no statistical significance in shRNA III group (p=0.1788), we think it may attribut to partail inhibition of PNAS-2, and it also shows a tendence of increased apoptosis ratio (7.19% while in shRNA control group is 3.52%). Over-expression study has showed cell apoptosis ratio statistical significantly decrease when PNAS-2 gene is over-expressed. We reveal the biologic effects of the expression level of the PNAS-2 transcript are associated with cell apoptosis, PNAS-2 is an anti-apoptosis gene.

1313

PNAS-2, A NOVEL GENE PROBABLY PARTICIPATE IN LEUKEMOGENESIS

H.R. Wang

RenJi Hospital, SHANGHAI, China

We use gene chip and find *PNAS-2* (Gene ID:AF229832) is one of the down-regulated genes upon treatment of As4S4 in APL cell line-NB4, same result has been reported by other group except the configuration of arsenic sulfide is As2S3 instead of As4S4. Moreover, PNAS-2 expression unchanged in U937 and K562 leukemia cell lines. The hypothetic protein of PNAS-2 shows high sequence similarity to a protein that is thought to be involved in apoptosis, however, there are no studies characterizing this gene. To obtain 5' unknown sequence of *PNAS-2* in NB4 cell line; To know whether *PNAS-2* is a pseudogene and its expression-spectrum both in multi-tissue and patients. 5'RACE was used to obtain 5' unknown sequence of *PNAS-2*; PNAS-2-GFP-fusion proteins express plasmid was constructed, after transfected to U937 cell line, Western blot analysis was applied to detect GFP fusion proteins; Northern Blot was used to detected the expression of *PNAS-2* gene in the multi-tissue; Real-Time PCR was applied to detected *PNAS-2* expression in patients.

After 5'RACE, we found two splice patterns of *PNAS-2* in NB4 cell lines, as F1 *PNAS-2* and F2 *PNAS-2*; both were more than 98% homology to *CHMP5*, *CGI-34* and *HSPC177*, these genes had a same open reading frame (Figure 1). After tranfected GFP fusion protein expression plasmid to U937 cells, we applied Western blot analysis. The results confirmed *PNAS-2* could be translated into protein and it was not a pseudogene (Figure 2). Northern Blot was applied in the multi-tissue including heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, lymph node, thymus, leukocyte, bone marrow and fetal liver, we found no expression of PNAS-2 gene in majority tissues except in placenta (Figure 3, 4).After Real-Time PCR, we found PNAS-2 expression statistically higher in 77 cases of acute leukemia (AL) include 71 *de novo* and 6 relapse when compare with 8 complete remission (CR) patients (p=0.0001) or 37 non-tumorous disease patients (p=0.0003). (Figure 5). There was no statistic difference between each subtype of AL.

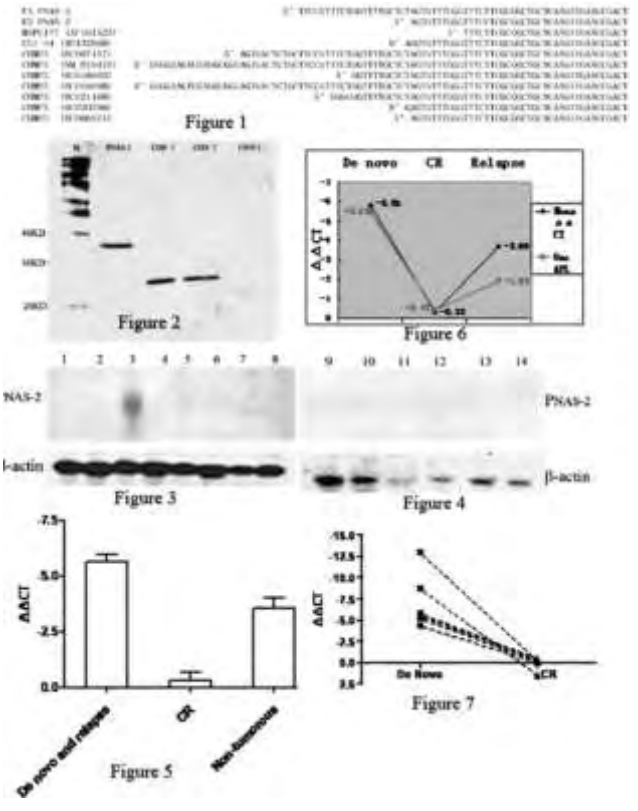


Figure 1. After blasted on website, the result showed both F1 PNAS-2 and F2 PNAS-2 were more than 98% homology to CHMP5, CGI-34 and HSPC177. Not only the ATG initial code of protein translation but also the open reading frame were completely same in these genes, indicated F1 PNAS-2 , F2 PNAS-2, CHMP5 , CGI-34 and HSPC177 were alias of the same gene as them had been presumed and they could be translated to same protein. Figure 2. Western blot analysis Result: M:marker; PNAS-2: cells trandfected with PNAS-2- pcDNA3.1/CT-GFP-TOPO vector showed a GFP-fusion protein was detected and it was about 30-40KD; con1 was transfected with Control 1-pcDNA3.1/CT-GFP-TOPO vector , a GFP-fusion protein about 20-30 KD had been detected; con2 was U937 cells which had been transfected with Control 2-pcDNA3.1/CT-GFP-TOPO vector and could observe a GFP-fusion protein about 20-30 KD; con3 was cells transfected with Control 3 pcDNA3.1/CT-GFP-TOPO vector, and could not find fusion protein. According to our design, the cells transfected with PNAS-2-pcDNA3.1/CT-GFP-TOPO vectors could express a PNAS-2-GFP fusion protein which is about 34 KD; transfected with Control 1-pcDNA3.1/CT-GFP-TOPO vectors can express a fusion protein consist of three histones, connective region peptide and GFP, which is about 28KD; transfected with Control 2-pcDNA3.1/CT-GFP-TOPO can only express a 26 KD GFP protein and U937 cells transfected with Control 3- pcDNA3.1/ CT-GFP-TOPO vectors can express neither GFP protein nor connective region peptide.This result coincided with our design, our findings confirmed PNAS-2 could be translated into protein and it was not a pseudogene.Figure 3,4. MTN hybridization Result: 1 heart; 2 brain; 3 placenta; 4 lung; 5 liver; 6 skeletal muscle; 7 kidney; 8 pancreas; 9 spleen; 10 lymph node; 11 thymus; 12 leukocyte ; 13 bone marrow, 14 fetal liver. PNAS-2 mRNA expressed only in placenta. Figure 5. Grouped t test showed PNAS-2 expression statistically higher in 77 acute leukemia patient de novo and relapsed compare with 8 complete remission (CR) patient (p=0.0001) or 37 non-tumorous diseas patients (p=0.0003); p value was 0.0019 between CR and non-tumorous disease patients. Figure 6. Analyse mean DCT of PNAS-2 manifest it highly expressed at diagnosis, remarkably decreased in CR stage and increased when relapse; data of one APL patient whom is under under observation in whole course coincided with this tendence. Figure 7. Paired t Test showed significant statistical difference of PNAS-2's expression between onset and CR in the same patient (p=0.0043), PNAS-2 decreased when these 6 patient achieved CR.

We found PNAS-2 highly expressed in 71 *de novo* AL patients compare with 8 CR patients (p = 0.0001); PNAS-2 also highly expressed in 6 relapse AL patients compare with CR patients (p=0.0166), but there was no statistical difference between *de novo* AL patients and relapse AL patients (p=0.0759)(Figure 6). We also found PNAS-2 expression noticeably decreased in 6 AL patients when achieved CR self-compared with onset of acute leukemia (p=0.0043) (Figure 7). We find F1 PNAS-2, F2 PNAS-2,CHMP5, HSPC177 and CGI-34 have a same ORF which indicates they can translate to same protein, and confirm these genes are alias of the same gene as have been presumed. PNAS-2 is not a pseudogene. Because no expression of PNAS-2 gene is observed in majority tissues, but it is remarkably up-regulated at disease presentation compare with non-tumorous patients and there is a relationship between PNAS-2 expression change and course of disease in AL patients: high expression at onset stage, decrease when achieve CR and increase again at relapse, it seems PNAS-2 gene may contribute to leukemogenesis.

1314

REVISIT OF DEL(20Q) IN MYELODYSPLASTIC SYNDROMES (MDS): RISK FACTOR ANALYSIS IN MDS PATIENTS WITH DEL(20Q)

K. Ohyahsiki,[1] Y.C. Liu,[2] Y. Ito,[1] H.H. Hsiao,[2] G. Sashida,[1] J. Ohyashiki[1]

[1]*Tokyo Medical University, TOKYO, Japan;* [2]*Kaohsiung Medical University, KAOHSIUNG, Taiwan*

The deletion of the long arm of chromosome 20, hereafter del(20q), is a common cytogenetic abnormality in various myeloid disorders and is known to be a favorable prognostic factor in myelodysplastic syndromes (MDS). However, del(20q) is sometimes found to be associated with disease progression and is detectable as one of additional cytogenetic changes. AIM: In order to ascertain the risk factors in MDS, we analyze 33 patients with MDS showing del(20q). We categorized del(20q) into two types; one is the sole and major del(20q) clone (>50% marrow metaphases) corresponding to genomic integrity, while the other is a late appearance of minor del(20q) clone (<50% metaphases) with additional cytogenetic changes representing genomic instability. Of the MDS patients with del(20q) at initial presentation, the negative factors in predicting prognosis on survival are (1) more progressive disease status, (2) any additional cytogenetic changes, or (3) minor del(20q) clone. CONCLUSION: The late appearance of del(20q) at any phase is linked to a significantly unfavorable prognosis, thus indicating the clinical and biological heterogeneity of del(20q) in MDS.

1315

THE 5'KIAA1509/3'PDGFRB FUSION GENE IN MYELOPROLIFERATIVE DISORDERS

L. Anelli,[1] A. Zagaria,[1] F. Albano,[2] R. La Starza,[3] A. Pannunzio,[2] V. Liso,[2] M. Rocchi,[4] G. Specchia[2]

[1]*University of Foggia, FOGGIA, Italy;* [2]*University of Bari, BARI, Italy;* [3]*University of Perugia, PERUGIA, Italy;* [4]*DI.GE.MI University of Bari, BARI, Italy*

Background. The myeloproliferative disorders (MPDs) are characterized by the abnormal proliferation of one or more myeloid cell types. Unlike the Philadelphia chromosome in chronic myeloid leukaemia, there is no specific chromosomal abnormality associated with the MPDs. However, a number of recurrent chromosomal rearrangements, involving a variety of tyrosine kinase genes as *PDGFRA*, *PDGFRB*, *FGFR1*, and *JAK2*, have been reported. In this report, we describe a patient with MPD bearing a t(5;14)(q32;q32). As a consequence of this rearrangement, the 5' region of the KIAA1509 gene was fused to the 3' portion of PDGFRB. *Aims*. We performed a molecular cytogenetic analysis by FISH to identify the genes mapping in correspondence to chromosomal breakpoints. Further molecular studies have been carried out to exactly define the breakpoints location within KIAA1509 and PDGFRB genes. The presence of this chromosomal translocation was investigated by fluorescence in situ hybridization (FISH) analysis on additional MPD cases. *Methods*. FISH experiments were performed with BAC clones specific for KIAA1509 and PDGFRB genes. The 5' KIAA1509/3' PDGFRB fusion transcript was detected using a KIAA1509 exon 11 forward primer (KIAA1509-11F) and a PDGFRB exon 11 reverse primer (PDGFRB-11R). The fusion protein domains were identified using the BLAST program (*http://www.ncbi.nlm.nih.gov/blast*). A FISH screening of 12 MPD cases was carried out. *Results*. FISH cohybridization experiments with RP11-368B7 and RP11-754J8 probes specific for KIAA1509 and *PDGFRB* genes revealed their involvement in the reciprocal translocation. RT-PCR analysis with KIAA1509-11F and *PDGFRB-11R* primers produced an amplification product of about 200 bp. The sequence analysis demonstrated that breakpoints were located within KIAA1509 intron 11 and *PDGFRB* intron 10. According to molecular data, the fusion protein was composed of 2 N-terminal KIAA1509 domains (coiled-coil myosin heavy chain tail and chromosome segregation ATPases region)

and a C-terminal *PDGFRB* domain (catalytic tyrosine kinase). The use of different primers combinations revealed the absence of the reciprocal 5' *PDGFRB/3'KIAA1509* fusion transcript. The FISH screening of 12 MPD patients with BAC clones specific for KIAA1509 and PDGFRB genes did not reveal the presence of other cases bearing the 5' KIAA1509/3' PDGFRB fusion gene. The patient with 5' KIAA1509/3' PDGFRB fusion transcript, was treated with imatinib and achieved hematological remission; the molecular response is still under evaluation. *Conclusions*. In this study we report the second MPD case with a t(5;14)(q32;q32) bearing a 5' KIAA1509/3' PDGFRB fusion gene. Our case differ from that previously reported in literature as KIAA1509 breakpoint was mapped within intron 11 instead of intron 9; any difference was observed in PDGFRB breakpoint location. As a consequence of this diversity, in our case a larger fusion protein was produced including an additional chromosome segregation ATPases domain. Treatment with imatinib resulted in hematologic response in both cases. Our data illustrate how molecular cytogenetic techniques may be useful to uncover recurrent chromosomal rearrangements in MPD patients.

1316

DETECTION AND MONITORING OF CYTOMEGALOVIRUS(CMV) IN BONE MARROW TRANSPLANT (BMT) RECIPIENTS BY REAL-TIME PCR (RQ-PCR)

N. Obeidi, H. Ghaffari, A. Gharehbaghian, K. Alimoghaddam, M. Dehghan, A. Ghavamzadeh, A. Shamshiri

Bushehr Medical University, BUSHEHR, Iran, Hematology-Oncology & BMT Research Center, Tehran University of Medical Sciences, Iran Blood Transfusion Organization Research Center, Iran

CMV has been recognized as the most important viral pathogen in persons undergoing BMT. Monitoring of CMV reaction from latency is critical for these patients. We could detect CMV DNA in this patients by RQ-PCR For monitoring of CMV reaction. If copy number of CMV was increased, preemptive therapy will be initiated. 51 recipients of BMT (9-51 years) were monitored as weekly intervals until day 100 after transplantation. For amplification of the pp65 gene (UL83) RQ-PCR assay and pp65 Antigenemia method were preformed in parallel with 415 samples. By cloning of this region, we made standards for RQ-PCR. The results obtained by the two techniques were significantly correlated (p<0.01). We could detected 13x101-15x107copies/2x10^5 cells by RQ-PCR.76% of patients developed more than one episode of CMV replication. First positive result of RQ-PCR 13 days earlier than the Antigenemia. After preemptive therapy 16 days (7-21 days) needed to become negative result of RQ-PCR. There was no relationship between death and increase of CMV copy(p<0.419). There is no correlation between copy number of CMV virus and PP65 and WBC count (p<0.624,p<0.422). RQ-PCR was more sensitive than pp65 Antigenemia. After preemptive therapy, negative results of RQ-PCR were the best indicator for determining of successful treatment. Reaction of CMV in our patients mostly endogenous and depend on kind of immunosuppressive therapy. If copy number of CMV increased one log, CMV reaction developed 1.22 fold.

1317

MONITORING OF MINIMAL RESIDUAL DISEASE AND TREATMENT OF MOLECULAR RELAPSES IN PATIENTS WITH ACUTE PROMYELOCYTIC LEUKEMIA

E.N. Shuravina, E.N. Parovitchnikova, I.A. Demidova, A.V. Misiurin, V.G. Savchenko

Russian National Center for Hematology, MOSCOW, Russian Federation

Backgrounds. Minimal residual disease monitoring of PML/RARA chimeric transcript is widely used method for detecting of molecular relapses (MR) in pts with APL during hematological remission. However, the necessity of therapy changing when MR is detected is still debated. *Aims.* We tried to find out whether the PML/RARA detection during hematological remission ultimately leads to relapse of APL and to develop the optimal treatment strategy of MR in APL pts. *Materials and Methods.* We investigated bone marrow samples by RT-PCR for PML/RARA chimeric transcript in 73 pts with newly diagnosed and morphologically proved APL. Primers synthesis for nested RT-PCR was performed using recommendations of BIOMED-1 Concerted Action (1999).). RT-PCR was performed on fresh marrow aspirates of all pts before treatment and periodically (2-3monthly) during all period of therapy (2 years after induction of remission). MR was defined as probable if chimeric transcript was detected once and was not find out by second investigation and as proved when *PML/RARA* was detected at least twice by consecutive investigations (in 2-4 weeks). *Results.* In 69 pts (94,5%) *PML/RARA* chimeric transcript was revealed during first investigation. 31 (45%) demonstrated bcr1 type of transcript, 38 (55%) - bcr3 type. In 4 pts (5,5%) *PML/RARA* was not found. During maintenance therapy in 19 of 52 pts (36,5%) MR was detected. In 5 patients from 6 with proved MR and in 3 pts from 13 with probable MR therapy was changed for Ara-C with idarubicine in early MR (12 months from remission induction) or ATRA + Interferon alfa in late onset of MR. No one of these pts developed hematological relapse. Maintenance was not changed in 11 pts (10 with probable MR, one - with proved MR) and 4 (36%) of them subsequently relapsed (one with proved MR). *Conclusions.* According to our data, detecting of *PML/RARA* in pts during maintenance therapy leads to high incidence of relapse in APL pts. Changing of therapy during MR significantly decreases the probability of hematological relapse [from 36% to 0% (p=0,001)].

1318

MONOCLONAL ANTIBODY TO CD34 INHIBITS PROLIFERATION AND INDUCES APOPTOSIS OF CD34+ STEM AND MYELOID CELL LINES

P.S.W. Stockbauer, K. Elknerov, J.S. Soucek, J.N. Nemcova, J. Schwarz

IHBT, PRAGUE, Czech Republic

Backgrounds. Monoclonal antibodies to epitopes of membrane differentiation antigens are widely used for therapeutical targeting of tumor cells. Aims. In some hematological malignancies, however, there is a need for more specific antibodies targeting the surface epitopes expressed on immature hematopoietic cells. *Methods.* We developed mouse monoclonal antibody of IgG1 class, clone 4H11, reactive with the class III (protein) epitope of human CD34 molecule. We detected the antiproliferative effect of CD34 antibody on human CD34+ stem and myeloid cell lines. Inhibition of proliferation was tested by uptake of tritiated thymidine and apoptosis was detected by Annexin-V-Fluorescence kit. *Results.* Anti-CD34 antibody 4H11 inhibited proliferation and induced apoptosis of CD34 positive cell lines at the concentration between 1-200 ug/ml after 12, 48 and 72 hours. The anti-CD34 antibody strongly inhibited proliferation and induced apoptosis of all CD34+ cell lines (MOLM-9, JURL, HEL, RPMI 8402) but not control CD34 negative cells. The antiproliferative effect was detected even at the antibody level of 2.5 ug/ml, and the antiproliferative effect was potentiated by simultaneous presence of differentiation inducing cytokines. The expression of CD34 antigen at the surface membrane of tested living cells was not modulated by 4H11 antibody. *Conclusions.* Based on the results obtained by the *ex vivo* model system of cultured leukemia cells we suggest that antigenic epitopes expressed on CD34 molecule should be considered as possible new molecular targets for the development of more effective targeted therapy of severe hematological malignancies, especially of immature myeloid lineage. (Supported by grant NR/8233-3 of the Internal grant agency of the Ministry of Health of the Czech Republic).

1319

ESTIMATION OF THE DIAGNOSTIC VALUE OF MYELOPEROXIDASE INDEX AND LDH IN MEGALOBLASTIC ANEMIA

J. Eivazi-Ziaei, S. Dastgiri

Tabriz Medical University, TABRIZ, Iran

Most cases of megaloblastic anemias corresponded to anemia with hyper-segmented neutrophils, macroovalocytosis and very high serum LDH level. Elevated neutrophil myeloperoxidase index (MPXI) may be indicative of a diagnosis of megaloblastic anemia. The aim of this study was to estimate the value of *MPXI* and *LDH* in the diagnosis of macrocytic anemia to facilitate the diagnostic algorithm prior to performing any bone marrow aspirate. *MPXI* and *LDH* were assessed using the first blood sample obtained prior to any transfusion or medical therapy, and after therapy in 29 patients diagnosed as megaloblastic anemia. MPXI was assessed using complete blood count (CBC), performed by Technicon H1 (Bayer) instrument. Mean value of MPXI significantly decreased after treatment (20.4, CI95%: 17-23 vs. -0.75, CI95%: -4-2.7, before and after treatment, respectively). The same significant pattern was also observed for LDH (4230, CI95%: 3096-5369 vs. 783, CI95%: 492-1075, before and after treatment, respectively). The proportional diagnostic value (%) was significantly higher when both MPXI and LDH (83 percent, p<0.001) were used together in the diagnosis of Megaloblastic Anemia while the same index was (71 percent, p<0.001) for MPXI and (48 percent, p<0.001) for LDH when they were used alone. MPXI and LDH values may have a diagnostic role on megaloblastic anemia. It might be used as a reliable screening tool before doing any other diagnostic procedure.

1320

INTERFERENCE OF *HBA1C* DETERMINATION BY THE HEMOGLOBIN VARIANT SHELBY

R. Scuderi,[1] T. Griffin,[2] S. Mehta,[2] D. Herold,[2] R. Fitzgerald[2]

[1]*University of California, San Diego, SAN DIEGO, USA;* [2]*Veterans Affairs Health Care Center, SAN DIEGO, USA*

Heterozygosity for Hb Shelby was serendipitously discovered in an asymptomatic 46-year-old African American male with a normal complete blood count following ion-exchange high-perfomance liquid chromatography (HPLC) determination of glycated hemoglobin (Bio-Rad Variant A1c). Aims. Characterization of the Shelby hemoglobin variant and consideration of its interference with HbA1c assessment. The patient had a recent history of blood glucose levels in the normal range (average value = 90 mg/dL), inconsistent with the measured HbA1c level of 12.9% [normal range 4.8-6.2%]. Of note, the interpretive software used for the A1c analysis identified the patient as having sickle cell trait (Hb A/S). Re-assessment of the degree of glycation using a boronate affinity column gave a more clinically appropriate value of 3.9% [normal range 4.0-6.0%]. Additional HPLC analysis using the β Thal Short Program (Bio-Rad) displayed an *unknown* peak comprising 26.3% of the total signal with a retention time of 4.84 minutes. Two previously described α-globin variants with similar retention times, O-Indonesia and O-Arab, displayed peaks with distinct conformational differences (slender peak bases versus a broad peak base) and associated glycation products not observed in our patient. Liquid chromatography-mass spectrometry (LC-MS) on a Finnigan LCQ using electrospray ionization showed a β-globin peak with a molecular weight of 15868 amu and a 738 amu shift from the α-globin peak, isobaric with the normal β-globin chain. Subsequently, all three exons of the β globin gene were sequenced bidirectionally at ARUP Laboratories. Heterozygosity for a nucleic acid mutation CAG→AAG at codon 131 (conferring a GLN→LYS amino acid substitution with zero mass change on MS), consistent with Hb Shelby, was found. This hemoglobin variant is described as unstable. Although asymptomatic, the patient did show a striking increase in the number of target cells on a peripheral smear. This report emphasizes the need to correlate laboratory findings with associated clinical parameters and to question results that do not appear reasonable. Determination of glycated hemoglobin by boronate affinity HPLC has been demonstrated as helpful in the monitoring of blood glucose control in patients with hemoglobinopathies.

1321

PREVALENCE OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN THE MALE POPULATION OF THE IRANIAN PROVINCE OF HORMOZGAN

M. Vakili,[1] M. Yavarian,[2] O. Safa,[1] G.R. Farshidfar[3]

[1]*Iran University of Medical Sciences, TEHRAN, Iran;* [2]*Shiraz University of Medical Sciences, SHIRAZ, Iran;* [3]*Hormozgan University of Medical Sciences, BANDAR ABBAS, Iran*

Backgrounds. G6PD deficiency or Favism is a red cell enzymopathy, which is very frequent in certain areas of the Persian Gulf and in African and Mediterranean countries. *Aims.* A relatively high incidence of G6PD deficiency and neonatal jaundice carried us to study the prevalence of G6PD deficiency in the Hormozgan province, an area in which about 1.2 million people lives. *Methods.* We randomly selected 816 male individuals aged between 15-20 years living in Bandar Abbas, Haji-Abad, Bandar Lengeh, Rodan, Minab, Jask and Geshm Island. The G6PD activity in the red cells was measured and classified in five categories, from I (the lowest) to V (the highest enzyme activity) and anemia level, according to WHO recommendations. *Results.* Our survey showed a heterogeneous variety of G6PD phenotypes: 3 cases (0.36%) in class I, 118 cases (14.46%) in class II, 134 cases (16.42%) in class III, 560 cases (68.62%) in class IV and 1 case (0.1%) in class V. The average hemoglobin levels in class I was 10.2 ±0.6 gm/dL and in other classes was within normal ranges. The geographical distribution of the prevalent rates was as below: class I (1.8%) and class III (18.5%) in the Geshm Island, Class II (23.9%) in the Haji-Abad, and class IV (77.1%) in the Bandar Lengeh areas. Our study showed that the mildest clinical symptoms (class IV) were found in the Bandar Lengeh area. The amount of NADP substrate needed to reach half of maximum reaction velocity (KM) was 9.1±7.15 (μmol/L) for class II; 3±0.9 (μmol/L) for class III and 3.6±1.8 μmol/L for class IV while the Km G6P for class II, III, and IV were 31.1±12.8; 44.8±10.7 and 50.5±12.5 μmol/L respectively. Except for class I carriers, who showed mild chronic anemia, the rest did not present any significant clinical symptom. However, some individuals had transient jaundice episodes in childhood. Hematological indices where also measured and a high percentage of the studied individuals (57%) presented with MCV lower than 80 fl. This is probably due to the high prevalence of α- and β-thalassemia traits and possibly iron deficiency in the areas. *Conclusion*. This study indicates that diagnosis and classification of G6PD deficiency should be routinely included in the public health care in the Hormozgan province. Moreover, further investigation is required for a better characterization of this disease at the molecular level.

1322

SEVERE IGA MEDIATED AUTOIMMUNE HAEMOLYTIC ANAEMIA IN HODGKIN'S LYMPHOMA PATIENTS: CASE REPORT

P. Moncharmont,[1] H. Ghesquieres,[2] C. Sebban,[2] P. Debourdeau,[3] M. Pavic,[3] P. Biron,[2] D. Rigal[1]

[1]*EFS Rhone Alpes Site de Lyon, LYON, France;* [2]*Centre Leon Berard, LYON, France;* [3]*Hia Desgenettes, LYON, France*

Backgrounds. In very rare cases a severe IgA-mediated autoimmune haemolytic anemia may be observed in Hodgkin lymphoma patients. Here is reported such a case. *Case report.* A 21-year-old man presented an asthenia with a dyspnea but without fever, loss of weight, pain, sweat, adenopathy, hepatomegaly nor splenomegaly - Two years before, a Hodgkin lymphoma with thoracic adenopathy and pericarditis had been diagnosed (Nodular sclerosis, stage II B). A chemotherapy including 6 AVBD cycles and a radiotherapy (30 + 10 Gy) had been performed and a remission had followed - At day (D) 0, an anaemia (Haemoglobin 87g/L, hematocrit 25.8%, and red blood cell (RBC) count 2.4 tera/L) was observed but without leucopenia nor thrombocytopenia. A hyperbilirubinaemia (48 μmol/L) was associated with a sharp drop in the haptoglobin level (lower than 0.2 g/L). There was a high rate of lacticodeshydrogenase (LDH) (1007 IU/L), but transaminases, C-reactive protein and fibrinogen levels were normal. A hemolytic anemia was suspected. Two D later, the anaemia was unchanged and the hemolysis confirmed, but the aetiology was not established. At D8, as the anemia was getting worse (Hemoglobin 75 g/L), new tests were carried out and a corticotherapy quickly started. The clinical course became satisfactory and no other immunosuppressive therapy or RBC transfusion were needed. *Methods.* RBC allo- and auto-antibody (Ab) were screened and identified by indirect (IAT) and direct antiglobulin test (DAT). The tests were performed using gel cards. In the DAT, anti-human IgG, IgM, IgA, C3c and C3d Ab were used. For the IgA auto-Ab testing, a second DAT with another anti-human IgA Ab was carried out by gel and tube methods. Results : For the first sample at D2 the IAT was negative and with the routine gel DAT, a negative result was observed with the anti-IgG and -C3d Ab. Whereas on the second sample at D8, the gel DAT performed was negative with anti-human IgG, IgM, C3c and C3d Ab but strongly positive with anti-IgA Ab. Using the second anti-IgA Ab, a strong positive reaction was also obtained in gel test, but negative in tube. Another gel test was carried out on the D2 sample but with anti-IgA Ab ; results were similar to those of the D8 sample. *Summary/conclusion*: IgA-mediated autoimmune haemolytic anaemia is rarely observed in Hodgkin lymphoma patients. When results are negative in the DAT with anti-human IgG, IgM, C3c and C3d Ab and the aetiology not established, a DAT with anti-IgA Ab is then recommended to detect these IgA RBC auto-Ab.

1323

α-THALASSEMIA CARRIERS IN CRETE: HEMATOLOGICAL AND MOLECULAR STUDY

D. Repapinou,[1] P. Karababa,[2] M. Boussiou,[2] V. Kafarakis[1], A. Papadaki,[1] A. Mavroudis,[1] D. Eliopoulos[1], A. Loutradi-Anagnostou[2]

[1]*University Hospital Heraklion, HERAKLION, Greece;* [2]*Thalassemia Center, Laiko Hospital, ATHENS, Greece*

Backgrounds. α-thalassemia is probably the most common monogenic disease. The prevalence of this disorder in different populations varies greatly ranging from less than 1% to over 90%. In the Greek population α-thalassemia appears to be quite heterogenic with frequency of carriers up to 8.3%, a percentage similar to that of α-thalassemia. *Aims.* To present the hematological and molecular findings of 69 α-thalassemia carriers in Crete, a region of Greece with increased incidence of α-thalassemia. *Methods.* Erythrocytic parameters and erythrocyte morphology were based on conventional methods, the chromatographic study was carried out with High Performance Liquid Chromatography (HPLC), while methyl-violet dye after incubation was used for the detection of HbH inclusions. Serum iron and ferritin concentrations were

determined with colorimetric and immunoenzymatic techniques, respectively. GAP PCR, and hybridization with allelic oligonoucleotides (ASO) were used for the molecular analysis for the most common α-thalassemic defects found in the Greek population, the deletional defects -$\alpha^{3.7}$,α^{MED}, α20.5 and the non deletional defects IVS1 5→pentanucleotide deletion, PolyA (AATAAA→AATAAG) and (AATAAA→AATGAA), Hb Agrinio and Hb Icaria. Statistical analysis was carried out with the Student's t-test. *Results.* Among the 72 α-thalassemic chromosomes of the 59 α^{+} and 10 α^{0}-thalassemia carriers, 44 (61.11%) deletional and 28(38.89%) non-deletional chromosomes were found. The deletions in the deletional chromosomes were the -$\alpha^{3.7}$ in the 37 chromosomes (84,09%), the α^{MED} in 6 chromosomes (13,64%) and the $\alpha^{20.5}$ in one chromosome (2,27%). The molecular defects in the non deletional chromosomes were the IVS1 5_pentanucleotide deletion in 23 chromosomes (82,14%), the PolyA TSaudi mutation in 3 chromosomes (10,72%) and the Hb Icaria mutation in 2 chromosomes (7,14%). All the non deletional defects were related to the β2-globin gene. Among the α^{+}-thalassemia carriers, MCV and MCH values were lower in IVS1 5→pentanucleotide deletion carriers than in -$\alpha^{3.7}$ deletion carriers (p<0.001 and p<0.001 respectively). No statistically significant differences were noted among the other erythrocytic parameters of these carriers. *Summary/Conclusions.* A higher percentage of non-deletional chromosomes, a higher percentage of the IVS1 5→pentanucleotide deletion and a lower percentage of the PolyA TSaudi mutation were observed in the α-thalassemia carriers in Crete compared to the previously reported percentages found in the general Greek population.

1324

APOLIPOPROTEIN E GENOTYPES IN IRANIANS WITH SICKLE CELL DISEASE

Z. Rahimi,[1] A. Vaisi Raygani,[1] R.L. Nagel[2]

[1]*Kermanshah Univ. of Medical Sciences, KERMANSHAH, Iran;* [2]*Albert Einstein College of Medicine, BRONX, NY, USA*

Background. Cardiac abnormality is one of complications in most of patients with sickle cell disease. Apolipoprotein E plays an important role in lipid metabolism. The apoEε4 allele has been known to be associated with risk of myocardial infarction and coronary artery disease. Aims. To determine the genotypes of apolipoprotein E and the frequency of apoEε4 allele in Iranians with sickle cell disease. *Patients and Methods*. Patients studied included 35 sickle cell anemia of which 21 were males and 14 females (aging 8-41 years), 15 sickle/β-thalassemia patients, 8 males and 7 females (aging 6-46 years) and 15 sickle cell trait individuals, 9 males and 6 females (aging 1- 58 years). Sickle cell phenotype was diagnosed using cellulose acetate electrophoresis at alkaline and citrate agar gel electrophoresis at acid pH and by solubility test. Hb A2 was determined by microcolumn chromatography method. DNA was extracted from whole blood using phenol-chloroform procedure. Apo E genotypes were analysed using PCR followed by digestion with Hha I restriction enzyme. *Results*. Of the six possible apo E genotypes, four were observed in sickle cell anemia patients that were ε3/ε3 (65.7%), ε3/ε4 (17.1%), ε2/ε3 (14.3%) and ε2/ε4 (2.9%). The frequencies of apo E alleles were: ε3 (81.4%), ε4 (10.0%) and ε2 (8.6%). In sickle cell trait individuals the order of frequencies of apo E genotypes was: ε3/ε3 (80.0%), ε3/ε4 (6.7%) and ε2/ε4 (13.3%). In sickle/β-thalassemia patients only two apo E genotypes (ε3/ε3, 86.7% and ε2/ε3, 13.3%) were existed. *Summary/conclusion*. It was concluded that the high frequency of apoE ε4 allele in Iranians with sickle cell anemia might increases the morbidity and mortality results from cardiac abnormalities in these patients.

1325

MOLECULAR CHARACTERIZATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN WESTERN IRAN

Z. Rahimi,[1] A. Vaisi Raygani,[1] H. Nemati,[1] R.L. Nagel,[2] A. Muniz[2]

[1]*Kermanshah Univ. of Medical Sciences, KERMANSHAH, Iran;* [2]*Albert Einstein College of Medicine,, BRONX, NY., USA*

Background. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a worldwide enzymopathy affecting an estimated 400 million people. Its presentation generally occurred as hemolytic episodes after ingestion of fava beans, unripe peaches, drug administration or infections. In many populations, the molecular defects responsible for this disease are one or a small group of mutations present at high frequency. *Aims*. To study the spectrum and frequency of *G6PD* mutations in school boys of Western Iran. *Methods*. The studied subjects were 64 *G6PD* deficient individuals comprised of 52 school boys aging 14-18 years diagnosed during schools screening and 12 children aging 1.5-13 years with history of favism and acute hemolytic anemia. All individuals were Kurds from Kermanshah province. DNA was extracted from whole blood by the phenol-chloroform method. Detection of mutations in coding region of *G6PD* gene was performed using PCR-RFLP analysis for the characterization of the *G6PD* Mediterranean and PCR-SSCP technique for the screening of exons 2 through 13. All mutation detected by SSCP were sequenced by an ABI system. *Result*s. The G6PD Mediterranean mutation (563 C:T) was detected in 57 males and one female, who was heterozygous for this mutation giving an allele frequency of 90.62%. *G6PD* Chatham (1003 G:A) in exon 9 was found in 5 males (7.81%). Nucleotide (nt) sequencing of exon 12 revealed a G:C substitution at nt 1376 (*G6PD* Cosenza) in one subject (1.56%). All but three individuals with *G6PD* Mediterranean mutation had T at nt 1311 (94.83%). *Summary/conclusion*. Our findings indicate that the allele frequency of *G6PD* Mediterranean mutation in Kurds from Western Iran is higher than those from two Fars ethnic groups living in Northern and Southern Iran. Nevertheless they are in strictly accordance with previous report of the prevalence of the *G6PD* Mediterranean in Kurdish and Middle East population. Also, the strong association of the *G6PD* Mediterranean mutation and the presence of the polymorphism nt-1311 C:T in the Kermanshah population demonstrate, that the presence of this mutation may be the result of migrations that have taken place through the history.

1326

ASSESSING ERYTHROPOIESIS IN HEMODIALYSIS PATIENTS; THE IMPACT OF PRO-HEPCIDIN LEVELS

C. Kartsios,[1] T. Eleftheriadis,[2] M. Ditsa,[3] C. Papadopoulos,[3] G. Antoniadi,[2] A. Skirta,[3] I. Stefanidis,[2] D. Markala[3]

[1]*'Theageneion' Cancer Hospital, THESSALONIKI, Greece;* [2]*Nephrology Dpt, University of Thessaly, LARISSA, Greece;* [3]*Hematology Dpt, Theageneion Hospital, THESSALONIKI, Greece*

Background and Aims. Prohepcidin (pro-HPC) is the precursor of hepcidin (HPC), a liver-derived peptide involved in iron metabolism by blocking its intestinal absorption and its release by the reticulo-endothelial system. Iron overload and inflammation increase HPC expression, whereas anemia and hypoxia suppress it. In the present study pro-HPC levels were determined in the serum of hemodialysis (HD) patients and its correlations with iron metabolism markers, C-reactive protein (CRP) and hematocrit (Hct) were assessed. *Patients and Methods.* 46 HD patients (M/F: 24/22, mean age: 61.1±12.8 years, mean time on HD: 52.2±48.5 months) and 22 healthy volunteers (M/F: 11/11, mean age: 52.2±19.2 years) were studied. Hct, serum pro-HPC, CRP, iron, ferritin, transferrin saturation and soluble transferrin receptors (sTFRs) were measured. Weekly erythropoietin dose and last month intravenous iron dose were recorded. *Results.* In comparison to healthy volunteers, HD patients had higher serum ferritin (359.77±96.87 vs. 67.58±48,27 ng/mL, p<0.001), sTFRs (0.465±0.173 vs. 0.307±0.109 mg/dl, p<0.001) and CRP (0.904±1.044 vs. 0.186±0.115 mg/dl, p<0.001), lower serum iron (64.45±33.3 vs. 99.77±41.62 ng/dl, p<0.001), Hct (34.1±3.14 vs. 43.35±3.7%, p<0.001) and similar transferrin saturation (28.83±13.33 vs. 31.81±11.59%, p:ns) and pro-HPC levels (257.46±96.87 ng/mL vs. 234.00±130.82ng/ml, p:ns). In the patients' group pro-HPC levels were negatively correlated with Hct (p: 0.022) but not with any other of the examined parameters. Multiple linear regression analysis considering age, inflammation, iron adequacy, erythropoietin dose and prohepcidin levels revealed that prohepcidin was the predominant determinant of Hct (p: 0.06). Conclusions. Taking into account the low Hct levels in HD patients of our study, it seems plausible that the pro-HPC levels assessed in this group are inappropriately high. These functionally high pro-HPC levels may belong to the factors that inhibit erythropoiesis in HD patients. On the other hand, the absence of other expected correlations indicates that further studies are needed in order to definitely clarify this aspect.

1327

ROLE OF ERYTHROPOIETIN IN THE TREATMENT OF PATIENTS WITH SEVERE HEART FAILURE

C. Lafaras,[1] E. Mandala,[2] D. Platogiannis,[1] G. Ilonides,[2] T. Bischiniotis[1]

[1]*Theagenion Cancer Hospital, THESSALONIKI, Greece;* [2]*D Department of Internal Medicine, Arist, THESSALONIKI, Greece*

Pathogenesis of anaemia in congestive heart failure (CHF) is multifactorial. Although the biological mechanisms linking anaemia and heart failure are not completely understood, prevalent anaemia is consistent

in patients with severe heart failure and is associated with higher mortality rates. Erythropoietin (EPO) promotes erythrocyte survival and differentiation and develops multiple paracrine-autocrine functions that coordinate local responses to injury. We investigated the effect of EPO administration in patients suffering from heart failure compatible with New York Heart Association functional classes III to IV. Twenty-four anaemic patients, 14 males, median age 62 years (range 47-77) were studied. Fifteen received EPO (15000 to 30000 IU per week) for three months. The rest of them comprised the control group. Heart failure functional class was comparable in both groups. Therapy included treatment with digoxin, angiotensin-converting enzyme inhibitors or AII blockers, carvedilol and diuretics and was not different between the groups. EPO was well tolerated by all patients. They underwent echocardiography in order to evaluate systolic and global left ventricular function. Ejection fraction (EF) and Tei index (calculated by dividing the sum of isovolumetric contraction and relaxation time by ejection time) were estimated at baseline and at the end of the study. Hemoglobin, creatinine and electrolytes were measured at baseline and every month later. Significant increase in hemoglobin values (10.2±0.5 g/dL to 14.2±0.7 g/dL, $p<0.01$) were observed in the EPO group, but no significant changes in the control group. Echocardiography showed improvement in left ventricular systolic and global function in the EPO group (EF 42±5% vs 48±6%, $p<0.01$, Tei index 0.58±0.14 vs 0.42±0.09, $p<0.01$), while echocardiographic indices remained unchangeable in the control group. 2/9 patients of the control group were hospitalized due to decompensation of heart failure and none in the EPO group. A slight decrease in creatinine values in the EPO group was detected at the end of the study, probably indicating improvement in renal vessels flow, but it was not statistically significant. EPO significantly improves systolic and global LV function, leading to increase of functional capacity and decrease of hospitalizations. Normalization of Hb concentration in patients with CHF may interrupt a vicious cycle, the recently coined cardio-renal-anemia syndrome. EPO may have a direct positive effect on the heart unrelated to correction of anaemia. Possible mechanisms could be prevention of tissue damage by reducing cell apoptosis and increasing neovascularization.

1328

THE IMPACT OF BONE FORMATION MARKER-OSTEOCALCIN-IN PATIENTS WITH β-THALASSEMIA

O. Salama,[1] Y. Al-Tonbary,[2] R. Shahin,[3] O. Sharaf Eldeen[3]

[1]*Clinical Pathology Department, Mansoura, MANSOURA, Egypt;* [2]*Pediatric Department, MANSOURA UNIVERSITY CHILDREN'S HOSPITAL, Egypt;* [3]*Clinical Pathology Department, MANSOURA UNIVERSITY, MANSOURA, Egypt*

The life expectancy of patients with thalassemia has greatly improved over the last years as a result of regular transfusions and increased compliance with iron chelation therapy, however, this improvement is often accompanied by a series of serious compliance including osteopenia and osteoporosis. The pathogens of these skeletal disorders is multifactorial which may be due to erythroid hyperlasia, increased iron stores or desferioxamine toxicity. The non invasive assessment of bone turnover has markedly improved with the development of specific and sensitive markers of bone formation. The aim of this work is to assess the value of some bone formation markers in patients with β-thalassemia. To achieve this goal 36 thalassemia patients were recruited in this study, they were 20 males (56.6%) and 16 females (44.4%) and their ages ranged from 3-18 years, beside 20 apparently healthy subjects of matched age and sex serving as a control group. The patients were selected from outpatient clinic and inpatient of Hematology/oncology Unit of Mansoura University Children's Hospital (MUCH). The selected subjects were subjected to thorough history taking, clinical examination, radiological evaluation and laboratory investigations including: complete haemogram, serum iron, serum ferritin, TIBC, serum calcium, phosphours and estimation of bone formation markers as alkaline phosphatase and osteocalcin. The results revealed that: serum calcium level was within the normal range and showed no statistical significance (p=0.176) when compared to control group while serum phoephours was significantly higher in thalassemic patients than control group (p=0.002) and this may be due to hypoparathyrodisim. Concerning the level of bone formation markers, serum alkaline phosphatase showed slightly higher level in patients than the control but this is not statistically significant (p=0.055), and this elevation can be referred to an associated liver disease in these patients. On the other hand osteocalcin level was significantly lower in patients than controls (0=0.011), and this may be refereed to osteoblast poisoning by iron overload. In conclusion, thalassemic patients have an unbalanced bone turnover between the bone formation and bone resoption markers and this is evidenced by non significant changes or decreased levels of bone formation markers while bone resorption is an active process.

1329

MOLECULAR ANALYSIS OF β-THALASSEMIA CASES IN POLAND

E. Zdebska,[1] A. Krawcewicz,[2] B. Burzynska,[3] M. Maciag,[3] U. Mokras,[2] J. Spychalska,[2] A. Adamowicz-Salach,[4] J. Koscielak[2]

[1]*Inst. of Haematology & Blood Transfusion, WARSAW, Poland;* [2]*Institute of Hematology and Blood Transf, WARSAW, Poland;* [3]*Institute of Biochemistry and Biophysics, WARSAW, Poland;* [4]*Medical Academy, WARSAW, Poland*

β-thalassemia is a heterogeneous, inherited disease resulting from reduced or absent synthesis of the β-globin chain of haemoglobin. This disorder is very common in Mediterranean, Middle Eastern, African and South East Asian populations. The aim of our study was to look for the mutations in the β-globin gene in the group of unrelated Polish patients with the β-thalassemia trait. 880 patients (396 men and 484 women) with microcytosis and no evidence of iron deficiency were examined for β-thalassemia. The Be Tha Gene 1 Analyte Specific Reagent (ASR) Module with the mDx Universal Module was used for detection of the 8 most common Mediterranean β-thalassaemia mutations in a patient's DNA sample (Bio-Rad Laboratories). The Be Tha Gene 1 test system is based on the principle of allele-specific oligonucleotide (ASO) hybridization. DNA Isolation Kit for Blood/Bone Marrow/Tissue (Roche Diagnostics GmbH, Germany) was used to isolate DNA from leukocytes. Polymerase chain reaction (PCR) was used to amplify the fragments of the β-globin gene Hemoglobin A2 was increased in 250 patients. In 130 patients there was also an elevation of hemoglobin F. 130 patients were examined for 8 common Mediterranean mutations. 7 different mutations were detected in 81 heterozygous patients (numbers of patients with a particular mutation are in square brackets): IVS1-6(T→C) [32]; IVS2-745(C→G)[25]; IVS2-1(G→A) [11]; IVS1-1(G→A) [4]; CD6 'A [2]; CD39(C→T) [4]; IVS1-110(G→A)[3]. DNA analysis revealed in two patients (of two unrelated families) with thalassemia intermedia mutation IVS1-6(T>C) in homozygote stage. Frequencies of individual mutations in Poland were different from those encountered in Mediterranean and some Central European countries.

1330

CONTRIBUTION OF MTHFR C677T AND A1298C SINGLE NUCLEOTIDE POLYMORPHISMS TO THE GENETIC SUSCEPTIBILITY OF SICKLE CELL DISEASE

W.Y. Almawi,[1] I.K. Al-Absi,[1] A.M. Al-Subaie,[1] G. Ameen,[2] K. Al-Ola[3]

[1]*Arabian Gulf University, MANAMA, Bahrain;* [2]*Bahrain Defence Forces Hospital, RIFAA, Bahrain;* [3]*Salmaniya Medical Complex, MANAMA, Bahrain*

Backgrounds. Methyletetrahydrofolate reductase (MTHFR) catalyzes the homocystiene-to-methionine conversion, and a reduction in its activity leads to elevation in homocysteine (Hcy) levels (hyperhomocysteinemia), a recognized risk factor for several thrombotic events. The MTHFR single nucleotide polymorphism (SNPs) C677T results in thermolabile enzyme and induces hyperhomocysteinemia, more than the A1298C SNP. Insofar as sickle cell anemia (SCA) is associated with a hypercoagulable state, many candidate genes were proposed to induce a prothrombotic state in SCA patients, including the MTHFR C677T SNP. Aims. This study addressed the prevalence of C677T and A1298C MTHFR SNPs among Bahraini SCA patients and control subjects, and correlate the genotype with changes in Hcy levels. *Method*: this was a case control study. Study subjects comprised 106 SCA patients (68 male and 38 female; mean age 15.8±9.8) and 165 healthy controls (80 male and 79 female; mean age 27.8±15.1); all were Bahraini nationals. Mutation analysis was assessed by PCR-RFLP analysis using Hinf I (C677T) and Mbo II (A1298C). Statistical analysis was performed on SPSS v. 13.0 statistics software. Fisher's exact test and Pearson's χ^2 test were used to assess inter-group significance, set at $p < 0.05$. *Results.* The frequencies of mutant T and C alleles of C677T and A1298C were comparable between patients and controls. Higher frequencies of the C/C variant of the A1298C but not C677T T/T (p=0.67) SNP was seen in patients than in controls (p=0.03; RR = 2.55). Differences between patients and controls in C677T and A1298C distribution were also noted in haplotype distribution. Elevated 677T/1298C haplotype was noted in patient (p = 0.05; OR = 2.589). While they were elevated in 677T/T (but not C/C) carrier, Hcy levels were comparable between patients and controls. Sum-

mary/conclusion: Results from this study showed that A1298C, but not C677T SNP, was associated with SCD. While the mechanism underlying C/C effect was not addressed here, it's not likely to involve changes in Hcy levels, since Hcy level was comparable between patients and controls.

1331

VITAMIN B12 DEFICIENCY AND THROMBOSIS

I. Yavasoglu,[1] G. Kadikoylu,[1] F. Yildirim,[1] J.A. Garcia-Vela[2]

[1]*Adnan Menderes University, AYDIN, Turkey;* [2]*QUIT study, BADALONA, Spain*

Backgrounds. Hyperhomocysteinemia is a risk factor for arterial and venous thrombosis. Acquired hyperhomocysteinemia may cause thrombosis in vitamin B12 deficiency. *Aim:* To evaluate the thromboembolic events in patients with vitamin B12 deficiency. *Methods.* One hundred forty-three patients (64 female, mean age 59±13 years) with vitamin B12 deficiency (vitamin B12 level < 200 pg/ml) were enrolled to this study. In control group, there were 129 healthy persons (62 female, mean age 58±8 years). Upper gastrointestinal endoscopy was performed to 102 patients. Antibody to parietal cells and the levels of homocsyteine were examined in 78 and 36 patients, respectively. In last three years, arterial and venous thromboembolic events were detected. χ^2 and student-t test were used in the comparison of two groups. Results. Thromboembolic events were detected in 9.8% of the of patients vitamin B12 deficiency. The sites of thromboembolic events were coronary arteries in 9 patients, deep venous and cerebrovascular thrombosis in two patients, respectively. There were thromboembolic events in 3.9% of controls. These rates were not different in two groups (p>0.05). The levels of homocsyteine were high (> 20 mMol/L) in all of 36 patients. *Conclusion*. Thrombosis was not higher in vitamin B12 deficiency. Although we did not examine the levels of homocsyteine in all of the patients, hyperhomocysteinemia may not contribute to thrombosis. More extended studies should be done in this topic.

1332

VALIDATION OF A DEDICATED HPLC METHODOLOGY FOR THE IMPLEMENTATION OF NEONATAL SCREENING OF HEMOGLOBINOPATHIES. A TOOL FOR PRIMARY AND SECONDARY PREVENTION IN A MULTI ETHNIC SOCIETY

M. Bouva,[1] C.L. Harteveld,[2] J.P.R. Doornbos,[3] P.H. Verkerk,[4] J.G. Loeber,[5] P.C. Giordano[2]

[1]*RIVM & LUMC, BILTHOVEN / LEIDEN, Netherlands;* [2]*Leiden University Medical Center, LEIDEN, Netherlands;* [3]*ZMC, ZAANDAM, Netherlands;* [4]*TNO Quality of Life, LEIDEN, Netherlands;* [5]*National Institute for Public Health, BILTHOVEN, Netherlands*

Backgrounds. Hemoglobinopathies (HbP's) are the most prevalent recessive disorder in Man. At least 300.000 affected children are born each year from parents who are both healthy carriers, most of them in endemic countries. Migrations have drastically changed the composition of the population in non-endemic countries where the incidence is growing. The number of patients in The Netherlands will double in the next decade if no prevention is offered. Although only partially effective for primary prevention the Ministry of Health recently decided to include the screening for hemoglobinopathies, and of sickle cell disease in particular, in the existing neonatal screening program. *Aims.* We have validated the Variant Newborn Blood Screening (Vnbs) HPLC apparatus (Bio-Rad) to determine if and how it could be used at a maximum of diagnostic efficiency in a national screening program for HbP. We intended to study how neonatal screening on HPLC could allow the implementation of secondary (morbidity) prevention to be planned in advance of the second semester of life, when the diseases will start manifesting. We also intended to study how primary retrospective and/or primary prospective prevention could be efficiently offered whenever an affected or a carrier neonate is detected and the parents are informed and referred to a genetic center for counseling and eventually prenatal diagnosis. *Methods.* We have created fresh artificial standard blood samples and we have used natural cord blood samples (CBS) to test the diagnostic confidence and the sample conditions before and after spotting aliquots on paper to be tested at increasing intervals of time up to a maximum of 3 weeks. Samples eluted from dry 3-mm paper discs were analyzed on HPLC, according to the manufacturer's instructions and in several modified manners. Results were compared with the expected patterns for their diagnostic quality and stability. *Results.* All current abnormal Hb's involved in SCD were identified using the artificial standard blood samples. In addition 94 natural CBS were analyzed on which we were able to identify HbBart's, and HbS traits. DNA analysis confirmed the association of HbBart's to a $-\alpha^{3.7}$ deletion. The samples spotted on paper degenerated rapidly. However, the interpretation of the results was still reliable on 15 days old dry samples, which period falls well within the boundaries of the screening program. The integration system of the Vnbs is not measuring the HbA% with the precision necessary to make an educated prediction upon a possible β-thalassemia minor. We are testing at this moment possible alternatives. *Summary/Conclusions.* The (Vnbs) HPLC apparatus recognizes with sufficient confidence all common Hb variants in heterozygous and homozygous state including HbS/S (SCD) and b-thalassemia major. This will enable pre-symptomatic genotype/phenotype determination and treatment planning for both diseases with a considerable gain in morbidity prevention and state of the art treatment. Moreover, obligatory or potential couple at risk can be immediately referred to a genetic centre for analysis, counseling and eventually primary prevention in a following pregnancy.

1333

A SIMPLE, ACCURATE METHOD FOR THE ESTIMATION OF THE ERYTHROCYTE SEDIMENTATION RATE

C. Lempesopoulos, K. Dina, G. Adamoulas, N. Ippikoglou, S. Paximadas, S. Kartas, N. Sakarelou

Elpis General Hospital, ATHENS, Greece

Backgrounds. measurement of the erythrocyte sedimentation rate (ESR) is a helpful indicator of the presence and extent of inflammation and its response to treatment. ESR is influenced by proteins of acute phase response and anemia which may be present in such situations. Aims. application of an easy, accurate, fast and low cost method for the estimation of the ESR. *Methods.* we studied 65 venous blood samples for ESR, 35 were taken from women and 30 from men. The method we used is that of Westergren as recom-mended by the International Council for Standardization in Haematology, and the anticoagulant we used was trisodium citrate in the proportion of 1 to 4. Regression analysis was used for describing the behavior of the ESR of the patients in an hour time period. Measurements of their ESR were recorded every ten minutes. Results. by using the control measurement of the ESR at 20 minutes, the blood samples were classified into three homogeneous groups (group-1: 0-5mmHg at 20 min, group-2 : 6-10 mmHg at 20 min, group-3 more than 10 mmHg at 20 min) and a family of regression curves was fitted to the empirical data describing the relationship of the ESR on time. The linear model was chosen as the simplest with good fitting precision in the study. Conclusion: the constructed linear curves within the established groups of the patients enable the estimation of the ESR values at 60 minutes period, with only one measurement at 20 minutes.

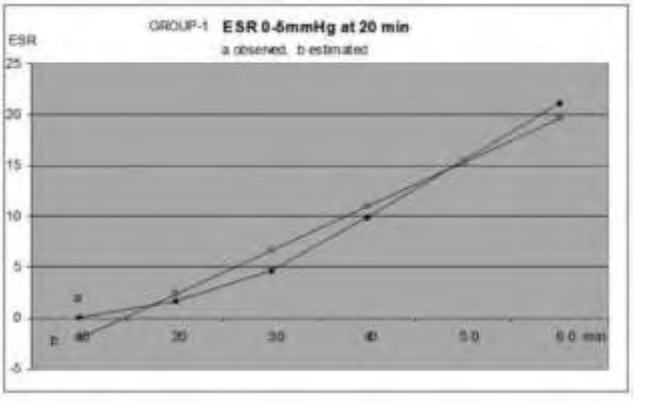


Figure 1. ESR1.

1334

NOVEL AND MEDITERRANEAN MUTATIONS IN β THALASSEMIA TRAIT INDIVIDUALS FROM NORTHERN IRELAND

M.J. Percy,[1] M. Knott,[1] K.M.A. Ramadan,[1] G. Savage,[2] F.G.C. Jones,[1] M. El-Agnaf,[3] M.F. McMullin[4]

[1]*Belfast City Hosptial, BELFAST, United Kingdom;* [2]*Royal Victoria Hospital, BELFAST, United Kingdom;* [3]*Ulster Hosptial, DUNDONALD, BELFAST, United Kingdom;* [4]*Queen's University, BELFAST, United Kingdom*

Backgrounds. Hemoglobinopathies, which include β thalassaemia, are a common group of genetic disorders prevalent in tropical and Mediterranean regions. They are co-incident with malaria suggesting that these disorders provide a selective advantage in malaria endemic areas. β thalassemia is characterised by deficient or absent synthesis of the β globin protein arising due to either point mutations, of which more than 200 have been described, or deletions of the β globin gene. Many of the common mutations are associated primarily with a particular population and haplotype analysis indicates they have common origins. Intriguingly, a low frequency of β thalassemia mutations have been described in non-tropical populations such as Britain and although most of these mutations are of non-native origin, some are novel. Previously several Irish cases of β thalassemia have been documented and a number of individuals with β thalassemia trait have been noted in the County Down region of Northern Ireland but the molecular basis in all cases has not been investigated. *Aims.* To discover if the β thalassaemia trait in County Down was associated with common or unique mutations and to perform haplotype analysis to indicate the origin of the common mutations detected. *Methods.* DNA samples from 23 individuals were screened for base changes in the β globin gene using PCR-direct sequencing. Haplotype analysis was performed using seven polymorphic sites of the β-globin gene cluster on chromosome 11. Markers were amplified by PCR and products were analysed by restriction digest. Haplotypes were constructed according to Orkin *et al*. [*Nature 296 (1982) 627*]. *Results.* Sequencing the β globin gene revealed that fourteen individuals possessed two common Mediterranean mutations, a C to T change at codon 39 in exon 2 and a G to A change at base 110 in intron 1. Both mutations were present on Haplotype I, indicating a non-native origin. A further group of seven individuals shared a G to A change at base 850 in intron 2. This mutation has been previously described in a American family of Scottish-English descent (*Curuk et al. Hematology 1995, 19:207*). Finally, two novel β-thalassaemia mutations were detected. The A to C change at the initiation codon would cause defective mRNA translation, while the deletion of G from the last base of codon 109 would result in frameshift mutation. *Summary*: The two Mediterranean mutations, having arisen on Haplotype I, are of non-native origin and may have been introduced into the Northern Ireland population as a consequence of European trade. It is probable that the IVS2-850 (G to A) detected in this study shares a common origin with the family described by Curuk *et al*. Finally, it remains to be confirmed if the novel mutations have arisen *de novo* in Northern Ireland.

1335

COMPARATION BETWEEN TECHNICON H3 AND COULTER GENS TECNOLOGY IN SPHEROCYTES DETECTION

M. Lopez Rubio,[1] C. Perez Calvo,[2] J. Garcia Suárez,[1] M.H. Bañas,[1] M.A. Calero,[1] I. Krsnik,[1] C. Burgaleta[1]

[1]Hospital Príncipe de Asturias, ALCAL DE HENARES, Spain; [2]Fundación Jimenez Diaz, MADRID, Spain

Spherocytes are not found in peripheral blood unless conditions such as hereditary spherocytosis, autoimmune haemolytic anemia, microangiopathy, haemoglobin C or Clostridium sepsis are present. The value of the quantification of MCHC by laser techniques or% Hyper (% of red cells with Hb concentration >410 g/l) as measured by Technicon cell counters has already been shown. When reticulocytes are measured by the Coulter Gens counter, the sample is subjected to a low osmolarity and a parameter known as MCVE is generated. Under normal conditions MCV is higher than MCVE but the reverse is seen when spherocytes are present. To determine if the measurement of MCVE is useful to detect spherocytes when compared to% hyper. To evaluate calculated CHCM by both counters and compare it with CHCM measured by laser (Technicon H3). We have simultaneously analysed 44 samples with a% Hyper >4 by both counters (Technicon H3 and Coulter Gens). Additional studies showed the following abnormalities in 23 of the samples: 11 were hereditary spherocytosis, 8 autoimmune haemolytic anemias, 2 hemoglobin C and one case of microangiopathy. Statistical analysis was performed with SPSS software, using Pearson correlation and receiver operating characteristic (ROC) curves. Parameters measured by both counters are shown in table attachment. The CHCM H3 values are bigger than calculated CHCM Gens. 'MCV-MVCE' median was positive. We have correlated% Hyper to the rest of parameters; the correlation was highly significative (p<0,002) with all the parameters but CHCM Gens. When sensibility and specificity of the techniques were evaluated using ROC curves, the values of area under the curve (AUC) were 0.808 for% Hyper and 0.766 for *MCV-MVCE*. There was significative correlation between the values of MCV-MVCE, measured by the Coulter Gens, and the presence of spherocytes. However, both sensibility and specificity of this technique were lower than that shown by '% Hyper' measured by the Technicon counter.

	Mean	*SD*
Calculated CHCM H3	35,68	1,33
Calculated CHCM gens	36,18	8,13
Laser-measured CHCM H3	36,85	1,41
% Hyper	12,25	8,78
MCV-MVCE	5,25	8,11

1336

WARM AUTOIMMUNE HEMOLYTIC ANEMIA (WAIHA) FOLLOWING RECURRENT MYCOPLASMA PNEUMONIA INFECTION IN A CHILD WITH DOWN SYNDROME

S. Bezirgiannidou,[1] D. Cassimos,[2] D. Pantelidou,[3] A. Chatzimichail,[2] M. Athanasiou,[4] G. Martinis[5]

[1]University hospital of Alexandroupolis, ALEXANDROUPOLIS, Greece; [2]Pediatric Departement, ALEXANDROUPOLIS, Greece; [3]Haematology Departement, ALEXANDROUPOLIS, Greece; [4]Aristotle University of Thessaloniki, THESSALONIKI, Greece; [5]Blood Transfusion Departement, ALEXANDROUPOLIS, Greece

Backgrounds. Cold agglutine syndrome (CAS) with cold IgM anti-I autoantibody represents a common hematological complication after Mycoplasma Pneumonia (MP) infection due to molecular mimicry. In only two cases both cold IgM and warm IgG autoantibodies have been identified. We present the first case of Autoimmune Haemolytic Anaemia (AIHA) following MP infection, with only warm IgG autoantibodies. *Case Report*: An 8 years old boy with Down Syndrome (DS), presented with MP infection. The patient in the last 2 years presented with recurrent episodes of MP infection, followed by non immune hemolytic anemia with DAT(-) and normal cold agglutinine titer. The patient was treated with clarithromycin. Four days after admission, laboratory findings of hemolysis were present: Hb 9.3gr/dl, Ht 29,6%, LDH 1200U/ml, reticulocyte count 5%, haptoglobulins 8mg/dl. Haemolysis was refractory to the initial treatment with α-globulin but was responded to prednizolone. The patient was followed up and presented additional MP infections, followed by WAIHA. The investigation of hemolysis was made by the ID-System of Dia-Med Company.The Blood group of the patient was A Rhesus(+) ccee, Kell(+), I antigen (+) positive. In the latest episode the immunohaematological results were as follows: Direct Antiglobouln Test (DAT): polyspecific(+)pos, anti-IgG (+)pos, titer anti-IgG: 1/100(+), anti-C3d(-)neg, anti-IgA(-)neg, anti-IgM(-)neg, anti-C3c(-)neg. Indirect Antiglobulin Test (IAT): (+) positive, owing to the presence of autoantibody. The serum and elution reacted with all red cells only at 37oC, in antiglobulin phase and the autoantibody was characterized as warm IgG panagglutinine while the cold agglutinine titer was < 1/64. The findings from DAT and IAT at the follow up were the same but the titer of anti-IgG was higher 1/400. Finally the elution study revealed an IgG autoantibody with anti-e specificity. Autoimmunity studies were negative for ANA, AMA, ASMA, anti-DNA, whereas antithyroglobulin was positive. The serum immuglobulines and the protein electrophoresis were normal. The CD4/CD8 ratio was low (0.45). IgM antibodies for MP were positive. However, antibodies for CMV, EBV, *Rubella*, HSV, VSV and Chlamydia were negative. *Summary/ Conclusions.* After MP infection, even with antibiotic treatment, the patient may remain chronic carrier. High incidence of MP infection and severe manifestation is observed in DS because of immune abnormalities like defective cellular adhesion and ineffective lymphocyte activation, due to indegrin (LFA-1) dysfunction. Excluding concomitant infections, drugs and hereditary factors, the fact that WAIHA was not a unique random episode but was recurrently following MP infections, indicates that MP may trigger WAIHA like other known autoimmune phenomena e.g asthma, Polyneyropathy, urticaria and Guillain Barre Syndrome. In DS, there are complex thymic alterations, that could be correlated with the presence of autoreactive CD4+T-cell, TH1/TH2 imbalance and early senescence of immune system resulting to early appearance of autoimmune phenomena like hypothyroidism, coeliac disease and IDDM. A polyclonal B-cell immune activation after a chronic MP infection in conjunction with a defective T-cell immunity that has been described in the genetic disorder of DS, may lead to WAIHA with warm IgG antierythrocytic autoantibody formation, instead of CAS that is usually seen post to MP infection.

1337

STUDY OF ERYTHROCYTE MEMBRANE PROTEINS BY SDS-PAGE 10 YEARS EXPERIENCE

M. Almeida,[1] L. Relvas,[2] U. Rebelo,[2] J. Vidán,[2] M. Ribeiro[2]

[1]*Hospital Pediatrico, COIMBRA, Portugal;* [2]*Centro Hospitalar de Coimbra, COIMBRA, Portugal*

Backgrounds. In the majority of Hereditary Spherocytosis (HS) and Hereditary Elyptocytosis (HE) cases the diagnosis can be made on the basis of clinical/ family history, red cell indices and osmotic fragility screening test. Quantification of erythrocytes membrane protein electrophoresis by SDS-PAGE can be useful in complex cases.

Group	Diagnostic information	n=	Protein reductions			
1	HS	321	Ankyrin/Sp./Pr 4.2	202	Band 3	115
2	HE	79	α+ β Spectrin	35	Pr 4.1	44
3	PK deficiency + HE	2	Pr 4.1	2		
4	α thal+ HE	8	α+ β Spectrin	3	Pr 4.1	2
5	β thal + HE	4	α+ β Spectrin	1	Pr 4.1	3
6	Myelofibrosis / HE	1	-			
7	HA unknown cause	48	Pr 4.1	4	Band 3	1
8	CDA	13	Band 3 qualitative alteration			2
9	EMPD	9	-			
10	SAO	2	-			
11	AIHA?	5	-			
12	Investigation of anemia	92	-			

HA - Hemolytic Anemia; CDA – Congenital Dyserythropoietic Anemia; PK - Pyruvate Kinase; thal - thalassemia; Pr - Protein; EMPD - Erythrocyte Membrane Permeability Diseases; SAO - Southeast Asian Ovalocytosis; AIHA - Auto Immune HA

Objectives. To identify the most common protein defects in our population and to analyze the usefulness of erythrocytes membrane protein electrophoresis in the diagnosis of patients with anemia and/or hemolysis of unknown or multiple etiologies, we made the retrospective analysis of 584 non familial cases. *Materials and Methods.* 584 EDTA blood samples from Centro Hospitalar de Coimbra and from other Hospitals in Portugal and Spain. SDS-PAGE was performed according to J. Delaunay protocols. The reasons for the study were divided in 12 groups as listed on the Table. *Results.* 63% of HS cases have combined Ankyrin/Spectrin/Pr 4.2 deficiencies, 34% combined Band 3/Pr 4.2 deficiencies and in 4% a single Pr 4.2 reduction was detected. Pr 4.1 reduction was found in 56% of the HE cases and the remainder 44% had Spectrin α+β reduction. In 14 cases with HA of multiple etiology we detected Spectrin α+β reduction in four and Pr 4.1 reduction in seven. Among the 48 samples with HA of unknown etiology, one was HS and four were HE. In 13 samples referred as possible CDAs, two had Band 3 abnormal mobility. In the EMPD, SAO and AHAI the electrophoretic profile was similar to the normal controls. No abnormalities were observed in the group of samples referring *investigation of anemia Conclusion*: In HS and HE the relative percentage of protein deficits involved are similar to the described for other European populations (*Delaunay et al., 1995, Eber et al., 1996*). In our experience, SDS-PAGE electrophoresis can be useful for the diagnosis of CDA type II, when an EMPD is suspected, in HA of complex etiology and when the results of screening tests are equivocal or borderline or the clinical phenotype is heterogeneous among the affected family members. If no spherocytes or eliptocytes are found in peripheral blood smears, erythrocytes membrane protein electrophoresis carries no benefit

1338

SPECTRUM OF ANTINUCLEAR ANTIBODIES IN SICKLE CELL DISEASE PATIENTS FROM OMAN

S. Al Kindi, A.V. Pathare

Sultan Qaboos University, MUSCAT, Oman

Backgrounds. Sickle cell disease [SCD] is a significant public health problem in the Sultanate of Oman. Although rare, it is not infrequent to find the SCD associated with systemic lupus erythematosus [SLE] or other connective tissue disorders. Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by a variable clinical picture, a large range of clinical and serological manifestations and a relapsing-remitting course. The disease is very variable in severity. The complexity of the clinical picture of SCD can be increased by the simultaneous presence of manifestations attributable to disease activity, chronic damage, co-morbidity, especially infection or the co-existence with SLE.

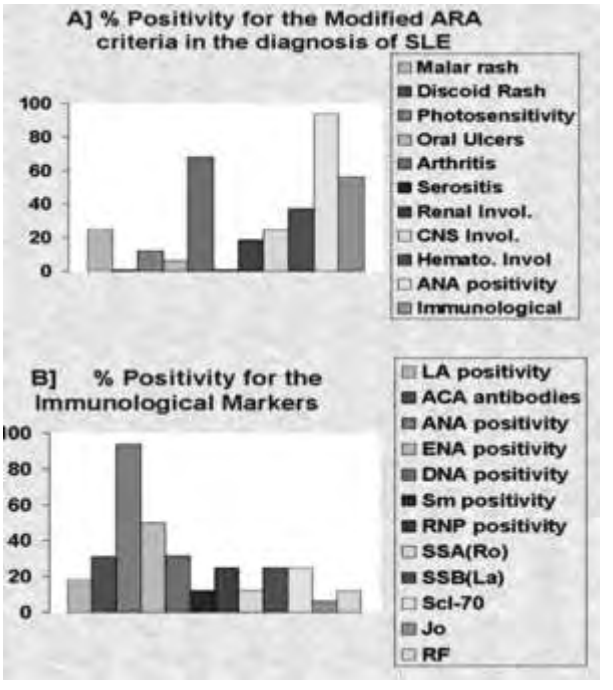


Figure 1. Clinical and Immunological characteristics.

Aims. To evaluate the prevalence of antinuclear antibody [ANA] positivity in sickle cell anemia patients. *Methods.* The study enrolled 67 SCD patients attending the Hematology services at the Sultan Qaboos University Hospital. All patients were explained the objectives of the study and gave an informed consent. Anticoagulated blood was collected for a full blood count and hemoglobin electrophoresis by high performance liquid chromatography. Blood was also collected for a battery of autoantibody profiles including anti-nuclear antibodies, double stranded anti-DNA antibodies and anticardiolipin antibodies, etc. All these tests were also performed in 107 healthy blood bank donors after informed consent, and in 35 sickle cell trait subjects as controls. *Results.* A total of 67 patients [31 males;36 females] with the mean + SD age of 24.6+7.9yrs[Range 11-47] formed the study group. ANA was documented to be positive in 16/67 cases [24%] amongst the SCD patients. ANA positivity was noticed in 10/107[9.35%] normal subjects and 6/35[17.2%] sickle cell trait subjects. 6 of the 16[37.5%] SCD patients satisfied the revised classification criteria [minimum 4] for SLE of the American College of Rheumatology.[Figure] Three patients had lupus anticoagulant, five had ACA positivity, one had Bechets, one had anti-thyroid antibodies and one had anti-red cell antibodies. *Discussion*: The study has demonstrates the prevalence of ANA positivity in normal subjects, sickle cell trait subjects and patients with SCD in Oman. The overall ANA positivity was observed to be about 24% which is considerably high. The prevalence was also twice as high in females as seen in the males. Furthermore, a significant number of these patients also had multiple autoantibodies.

1339

LYMPHOID EXTRAMEDULLARY BLAST CRISIS OF CHRONIC MYELOID LEUKAEMIA SIX YEARS AFTER ALLOGENIC TRANSPLANTATION

A. Garcia-Noblejas, A. Durán, M.E. Fernandez, A. Acevedo, R. de la Camara

La Princesa, MADRID, Spain

Extramedullary disease (EMD), also called granulocytic sarcoma, following allogeneic hematopoietic stem cell transplant (allo-HSCT) in patients diagnosed with chronic myeloid leukaemia (CML) is an infrequent event. According to a retrospective analysis performed by the European Group for Blood and Marrow Transplantation the incidence rate for this complication was 0.22%. Considering that lymphoid transformation accounts for only 20-30% of all blast crisis events in CML, lymphoid EMD remains a relatively rare phenomenon. Reviewing the literature we found only four cases of lymphoid EMD relapse after an

allo-HSCT. We present here a new case of lymphoid EMD relapse six years after an allo-HSCT. A 60-year old woman was diagnosed with Ph-positive CML in accelerated phase in 1.998. Ten years before she was treated for a breast cancer with surgery, radiotherapy and chemotherapy. After an initial treatment with hidroxyurea and interferon-α, she received an allogenic HSCT from her HLA matched brother in January 1.999, conditioned with oral busulfan and cyclophosphamide (BuCy2). Ciclosporine A and methotrexate (days +1, +3, +6, +11) were given as prophylaxis for graft-versus-host-disease (GVHD). Post-transplant she obtained a complete donor chimerism and complete molecular response of her CML, and developed a mild acute skin GVHD followed by an extensive moderate chronic GVHD. Inmunosuppresive regimen ended in September 2.003. In September 2.005, more than 6 years after HSCT, she presented with a progressive right lower limb pain, weakness and intermittent numbness. Areas of increased uptake in right pelvis were seen in the γgraphic study with Tc-99. A big osteoblastic lesion in right pelvic (from right iliac fosse to acetabulum) with a surrounding mass involving the gluteus and ilio-psoas muscle was detected by a magnetic resonance study. Laboratory studies showed a positive RT-PCR for Bcr-Abl in peripheral blood and bone marrow but with a normal masculine karyotype and negative FISH evaluation for Bcr-ABL rearrangement. A bone marrow chimerism study revealed 1% of autologous cellularity. A surgical biopsy of the lytic lesion showed a massive blast cell infiltration with the phenotype: CD34 +, CD45 weak, CD79a +, CD43+, Ki-67 + (>50% cells), Tdt +, CD 20-, mieloperoxidase -, CD 68 -. An isolated lymphoid extramedullary relapse was diagnosed. The patient was treated with local radiotherapy (total dose of 2.400 cGy), chemotherapy with HyperCVAD protocol and Imatinib mesylate. After 68 days the patient was pending on an image revaluation of the mass and lytic lesions. RT-PCR for Bcr-Abl in bone marrow has now become negative. We present a new case of lymphoid EMD after 6 years of an allo-HSCT, which represented the latest lymphoid EMD case reported until. As it is a very rare phenomenon, there isn´t a consensus about the best treatment. Usually local (surgery or radiotherapy) and systemic (chemotherapy, donor lymphoid infusion, imatinib mesylate) treatment is administered although the prognosis for these patients is very poor due to cumulative toxicities from the previous conditioning regimens and the resistant disease.

1340

SIDEROBLASTIC CHANGES CAN BE RECOGNIZED IN THE ERYTHROGRAM

A. Rovó, G. Favre, J. Passweg, D. Heim, S. Meyer-Monard, D. Tsakiris, A. Gratwohl, A. Tichelli

University Hospitals Basel, BASEL, Switzerland

New generation hematology systems supply essential information on blood cells (BC) complementing microscopic cell examination. The scatter plots of red blood cells (RBC), called erythrogram produced by the ADVIA 2120 cell counter gives a visual representation of RBC characteristics. Using a pair of threshold gates on each axis, nine areas are defined according to the cell volume (fl) and the hemoglobin contents (g/dl). Normal RBC are distributed in the central quadrant. The number of cells outside these thresholds gives an accurate percentage of macrocytic, microcytic, hypochromic and hyperchromic RBC. In diseases such as iron deficiency and thalassemia the erythrogram shows a characteristic pattern and is commonly used for diagnostic approach. We observed in patients, mainly with myeloid neoplasia with ringed sideroblasts (RS) in the bone marrow a particular erythrogram pattern with a broad distribution of the RBC, a marked variation in RBC size and hemoglobinization. From the central quadrant an abnormal RBC population shifts on an imaginary axis to the lower-left quadrant representing a tail that advances into the microcytic and hypochromic quadrants (Figure 1). To confirm whether this particular erythrogram was predictive for bone marrows sideroblastic changes; we compared retrospectively the erythrogram of patients with RS to a group of myeloid neoplasia without RS. Between January 2004 and August 2005, 33 of 1973 cases with more than 15% of RS in the marrow iron staining were identified. In 12/33 cases the erythrogram was not available, 21 cases were evaluable (AML=2, MDS=13, MPS=1, MDS/MPS=3, non neoplasia=2). Patients were compared to 30 consecutive cases with myeloid diseases without RS (No RS) (AML=16, MDS=11, MPS=2, MDS/MPS=1). In addition to the erythrogram pattern, hemoglobin, RBC indices, and bone marrow iron staining were analyzed in both groups. We defined two types of erythrogram pattern in respect of sideroblastic changes: a) typical (figure 1) b) non typical: any other pattern (i.e. macrocytosis in myeloid diseases without RS.

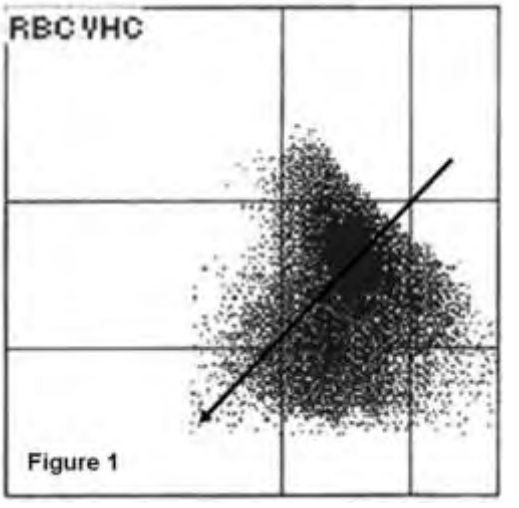


Figure 1

The erythrogram was typical in 17/21 patients with RS and in 0/30 patients with No RS ($p<0.0001$).The positive predictive value for sideroblastic changes was 100% and the negative predictive value was 88%. Despite the RBC indices comparison showed statistical significance in some variables, they were no specific enough to identify sideroblastic changes. In the group with RS, mean cell hemoglobin was lower (median 30.8 versus 33.6 fl), RBC distribution width was higher (19.3% versus 16.5%), the percentage of hypochromic RBC was higher (5.3% versus 0.9%) and hemoglobin content of reticulocyte was lower (33 versus 37 pg) as compared to No RS patients ($p<0.05$). This last index was useful to rule out iron deficiency in RS group as a cause of hypochromic RBC changes, since in contrast to iron deficiency it was not decreased. In conclusion: Sideroblastic changes can be recognized in the erythrogram. Indeed, despite myeloid neoplasia with RS are a heterogeneous group of diseases they have a common pattern of RBC distribution that can be considered as a kind of fingerprinting for sideroblastic changes with a high predictive value allowing a straightforward diagnostic approach in clinical practice.

1341

EPIDEMIOLOGICAL DATA ON MYELODYSPLASTIC SYNDROME PATIENTS FROM A ROMANIAN SINGLE CENTER

R. Gologan, D. Georgescu, A. Tatic, I. Radulescu, D. Vasilache

Fundeni Clinical Institute, BUCHAREST, Romania

Background. Since the World Health Organization (WHO) recognized MDS as a disease entity only starting with 1997, epidemiological data on MDS cannot be obtained from official statistics on morbidity and mortality and have to be extracted from specialized registers. We present the first romanian study on the incidence and characteristics of MDS, based on the data existing in Fundeni Clinical Institute, Bucharest, the greatest hematological department in Romania. *Method*. The MDS files at diagnosis of the patients admitted during the period 1980-2005, recorded in the registration forms provided by the MDS Foundation (USA), represented the primary data-base. The hematological data of the MDS patients included in the registry were re-evaluated and classified according to French-American-British (FAB) criteria. The distribution by sex, age groups, subtypes and the annual number of new cases were analysed comparatively with other reference studies. *Results*. Four-hundred and twenty four cases of MDS were identified. The distribution between sexes was relatively balanced with a slight global preponderance of males ((M/F 1.26), except for refractory anemia with excess of blasts (RAEB) 1.94. The mean age at diagnosis was 62.3 years (16-90). Most of the patients (60.6%) belonged to the group of age 61-80, where all the subtypes of MDS had the highest rates. A noticeable proportion (17%) had ages below 50 years, 25% of which in the range 16-30. On the other hand, few cases (4%) were above 81. Patients with refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) accounted for 44.5% of all cases (RA 29%, RARS 15.5%), RAEB and RAEB in transformation 33%, chronic myelomonocytic leukemia 5.6% and unclassified 16.7%. The annual number of new cases was constantly low during the period 1980-1989, but increased dramatically from 11 cases/year in 1990 to a maximum of 48 cases/year in 1999, showing a

certain decrease afterwards. The subtypes with the most important increase in time were RA and RARS. *Conclusions*. This study indicates an actual increase of the number of MDS cases in Romania over the investigated period of time. Particularly, a noticeable proportion of young patients and a low proportion of patients ≥ 81years have been found, which make our findings closer to the Asian than to the Western MDS epidemiological results.

1342

SINGLE PEGFILGASTRIM INJECTION AFTER FLUDARABINE-CYTARABINE BASED REGIMENS FOR TREATMENT OF POOR PROGNOSIS MYELODISPLASTIC SYNDROME (MDS) AND ACUTE MYELOID LEUKEMIA (AML): PRELIMINARY DATA ON HAEMATOLOGIC RECOVERY

M. Tassara, A. Crotta, L. Camba, F. Lunghi, M. Marcatti, J. Peccatori, F. Ciceri, M. Bregni, M. Bernardi

San Raffaele Scientific Institute, MILAN, Italy

Backgrounds. response of poor prognosis MDS and AML to conventional chemotherapy (CT) is unsatisfactory. Regimens comprising Fludarabine and Cytarabine (FLA), with or without Idarubicin, have shown promising results in induction of complete remission (CR), with a favourable toxicity profile. In FLA regimens filgrastim is administered from day 0 to day 5 to induce cell cycling and therefore sensitization to CT, then from day 12 to enhance recovery of neutrophils. Peg-filgrastim is a covalently bound conjugate of filgrastim and monomethoxypolyethylene glycol. It has a longer elimination half-life than the unconjugated filgrastim because of decreased serum clearance. After standard chemotherapy for non-myeloid malignancies, one dose of Peg-filgrastim showed to be equivalent to daily filgrastim in enhancing neutrophil recovery, and the single injection was largely preferred by patients (pts). From march 2005, in MDS/AML pts we started to administer a single dose of Peg-filgrastim at day 12 of FLA regimens instead of daily unconjugated filgrastim. *Aim*: to evaluate the efficacy and cost effectiveness of a single Peg-filgrastim injection given at day 12 from the beginning of FLA regimens, in poor prognosis MDS and AML pts. *Methods.* from March 2005 to December 2005 13 FLA cycles with Peg-filgrastim s.c. injection at day 12 have been administered to 10 pts, at our Institute (Group PEG); neutrophil and platelet absolute count have been monitored daily from day 0. Data on haematologic recovery after 53 FLA cycles with unconjugated filgrastim (dosage: 300 mcg/sqm/day) in 36 pts, period January 1999-February 2005, have been retrieved from our database (Group NO-PEG). Filgrastim has been administered until neutrophil count > 500/mmc. Group PEG: median age 66 (range 49-73); diagnosis of MDS=3, AML=6, granulocytic sarcoma=1; status pre-FLA: CR1= 5, CR>1=2, NOCR=6. Group NO-PEG: median age 56 (range 22-69); diagnosis of MDS=22, AML=14, granu zlocytic sarcoma=0; status pre-FLA: CR1=16, CR>1=0, NOCR=37. *Results.* data on haematologic recovery are shown in the table.

Table 1.

	Group PEG		Group No PEG	
	median	range	median	range
neutrophils <500/mm³ (n° of days)	17	7-27	16	8-37
neutrophils >500/mm³ (day from start of CT)	20	18-37	20	14-39
Platelets <20000/mm³ (n° of days)	18	4-n.e.	19	8-n.e.
Platelets >20000/mm³ (day from start of CT)	22	18-n.e.	23	11-n.e.

n.e.=not evaluable.

A single injection of Peg-filgrastim has been administered in 12 out of 13 cycles in Group PEG; in one case a second injection has been administered at day 32, for delayed recovery. The mean number of vials per cycle of unconjugated filgrastim administered to Group NO-PEG has been 21 (range 6-57). *Conclusions.* according to our preliminary results, a single Peg-filgrastim injection after FLA regimens results in a haematologic recovery comparable to that achieved by daily unconjugated filgrastim; therefore, it would safely spare patients to receive multiple injections. Moreover, regarding the high number of filgrastim vials required to enhance neutrophil recovery in our pts, the conjugated formulation could be favourable also in terms of cost-effectiveness. These preliminary data must be confirmed in a larger population of pts.

1343

ANALYSIS OF CASPASES GENES EXPRESSION IN THE BONE MARROW OF ADULT *DE NOVO* MYELODYSPLASTIC SYNDROMES (MDS)

C. Economopoulou

Attikon University General Hospital, ATHENS, Greece

Backgrounds. Myelodysplastic syndromes cover a range of clonal stem cell disorders characterized by ineffective hematopoiesis which has been associated with excessive intramedullary apoptosis of hematopoietic cells. Caspases constitute a family of cytosolic proteases which are the effector molecules of apoptosis. *Aim*: The aim of the present study was to examine caspases and granzyme B expression and the degree of apoptosis in the bone marrow of adult de novo myelodysplastic syndromes and to correlate our findings with clinical parameters and prognosis. *Methods*. We studied 31 cases of MDS including 7 RAEB-t, 9 RAEB, 4 CMML, 7 RA and 4 RARS according to FAB criteria. The degree of apoptosis was determined by flow cytometry using the Annexin method on fresh bone marrow mononuclear cells. mRNA was extracted and the expression of caspases 1, 2 , 3,5,6,7,8 and 9 and Granzyme-B was determined using a multiprobe RNase Protection Assay System (Riboquant, BD Biosciences). A pool of RNA from normal bone marrow mononuclear cells was used as a normal control. The expression of each gene was compared to that of two housekeeping genes (GAPDH and L32) using the Image Master analysis Software . The level of each gene expression was compared to that obtained from the normal pool RNA. A ratio was obtained and two groups were generated with values > or <1. The expression of the genes and the degree of apoptosis were analyzed taking into consideration haematological parameters, the FAB classification and the IPSS value. *Results.* The median value of apoptosis for all MDS cases was 4,7. Apoptosis in the low risk group was higher but not significantly different from the high risk group (8.9 in the RA-RARS vs 4.3 in the RAEB, RAEB-t and CMML). Caspase 8 was expressed in 14/31, caspase 3 in 15/31, caspase 6 in 15/31 , caspase 5 in 7/31,caspase 2 in 12/31, caspase 7 in 18/31, caspase 1 in 16/31 and caspase 9 in 14/31 cases including all FAB subtypes with the exception of caspase 5 that was not expressed in any of the 7 RA cases examined. Granzyme B was expressed in 18/31 cases. The level of expression as well as the percentage of positive cases for all genes examined was not significantly different between different FAB subgroups and different IPSS risk categories. The level of caspases and granzyme B expression did not correlate with different hematological parameters or the values of apoptosis. Moreover cases with ratio of gene expression compared to normal pool >1 vs those <1 did not differ significantly for all hematological parameters, the IPSS risk category, the FAB subtype and the level of apoptosis. *Conclusion*. Caspases1,2,3,5,6,8,9 were expressed in the bone marrow of adult *de novo* MDS in 38-58% of cases including all FAB subtypes. Caspase 5 was less frequently expressed and was negative in all RA cases examined The level of caspases and granzyme B expression did not correlate with different hematological parameters, FAB classification, the IPSS risk group and the level of apoptosis. Larger number of cases need to be examined to draw definite conclusion about the role of these apoptosis regulatory genes in the pathogenesis and prognosis of MDS.

1344

THE SIGNIFICANCE OF LYMPHOCYTES PHENOTYPE IN MYELODYSPLASTIC SYNDROMES AND MYELODYSPLASTIC/MYELOPROLIFERATIVE DISORDERS

M. Vikentiou,[1] K. Psarra,[2] V. Kapsimali,[2] E. Grigoriou,[2] M. Michael,[2] E. Nikiforakis,[2] C. Papasteriades[2]

[1]*Kapodistrian University of Athens, ATHENS, Greece;* [2]*Evaggelismos General Hospital, ATHENS, Greece*

Backgrounds. Myelodysplastic Syndromes (MDS) represent a heterogenous group of hematological disorders diagnosed, so far, by morphologic and clinical findings supported by cytogenetics. However, these methods are inadequate to give definite diagnosis to all cases. For this reason many researchers are investigating the bone marrow (BM) and peripheral blood (PB) immunophenotyping of the MDS patients as an additional diagnostic tool. *Aims.* The purpose of the present work was to study

BM lymphocytes of patients with MDS, as there is a controversy between research groups regarding the lymphoid lineage participation in the pathogenesis, diagnosis and/or in the prognosis of MDS. Following the new classification of MDS by the World Health Organization (WHO) it seems challenging and interesting to investigate, apart from MDS, a group of patients with CMML, which has been classified by WHO as the new group of Myelodysplastic/Myeloproliferative Disorders (MDS/MPD). *Method*: BM samples from 32 patients with MDS (n=13), MDS/MPD (n=12), and MDS/AL (n=7) and 5 BM from healthy individuals, as a control group, were analyzed by multiparametric 3-color flow cytometry using an extensive combination panel of monoclonal antibodies (CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11b, CD11c, CD13, CD14, CD15, CD16, CD19, CD20, CD33, CD34, CD36, CD38, CD56, CD64, CD66b, CD79a, CD117, HLA-DR, KORSA, MPO/LF and TdT) in the gate of the lymphocytes by the histogram of SSC=f (expression of CD45). *Results.* The MDS patients were characterized by the following statistical significant findings in comparison to the control group: a) a decrease of the lymphocytes mean SSC (59,42±5,62 vs 87,60±22,52, p=0.001) and b) a decrease of the fluorescence intensity of CD45 in lymphocytes (43,98±15,99 vs 65,64±17,12, p=0.035). The MDS/MPD patients compared to the control group were characterised by: a) a decrease of the CD38+ (31,01±23,87 vs 42,34±25,06, p=0.013) and b) a decrease of CD56+ (5,80±6,69 vs 20,45±16,72, p=0.036) lymphocytes percentage. The MDS group in comparison to the MDS/MPD group showed a statistical significant decrease of the lymphocytes mean SSC (59,42±5,62 vs 70,83±17,02, p=0.014) along with a decrease of the percentage of co-expression of CD3/CD16/CD56 (5,80±3,86 vs 11,35±6,72, p=0.026). When MDS/MPD group was compared with MDS/AL a decrease of T lymphocytes (CD2+: 83,59±3,30 vs 88,06±8,37, p=0.039) and an increase of B lymphocytes (CD20+: 12,73±5,23 vs 7,34±6,08, p=0.014 and CD19+: 12,67±3,55 vs 7,36±5,64, p=0.039) were observed. It should be noted that myeloid markers expression in the lymphoid populations groups didn't show any differences. *Summary/Conclusions.* The above-mentioned statistical significant findings indicate the importance of further study of this cell lineage in MDS and MDS/MPD cases to answer several questions such as is the decrease of lymphocytes side scatter and CD45 expression indicative of lymphocyte immaturity? As this is an ongoing study, more cases will possibly clarify the disturbances of lymphocytes and their significance in this group of patients.

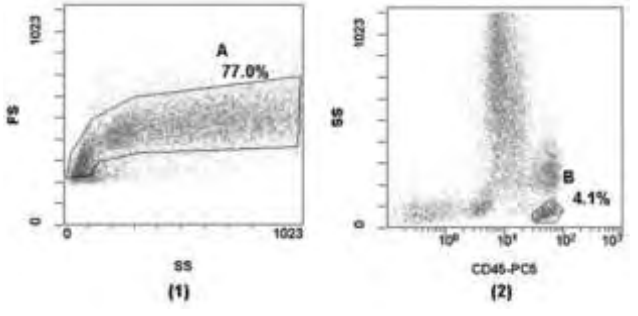


Figure 1. Lymphocytes gating of MDS/MPD BM sample.

1345

EPIGENETIC ALTERATIONS IN DLK1/GTL2 IMPRINTING STATUS IN MYELODYSPLASTIC SYNDROMES; PRELIMINARY RESULTS

L. Benetatos,[1] A. Dasoula,[2] E. Hatzimichael,[1] I. Georgiou,[3] E. Kapsali,[1] K. Bourantas,[1] M. Syrrou[2]

[1]*Medical School, University Hospital of I, IOANNINA, Greece;* [2]*Laboratory of General Biology, IOANNINA, Greece;* [3]*Laboratory of Genetics, Medical School, IOANNINA, Greece*

Backgrounds. Recently, an imprinted gene cluster at 14q32 has been defined and includes two closely linked but reciprocally imprinted genes, DLK1 and GTL2. DLK1 is a paternally expressed gene, encoding a cell surface transmembrane protein containing six epidermal growth factor repeats, while GTL2 is an untranslated maternally expressed gene. Loss of imprinting (LOI) is the loss of normal allele-specific gene expression through the disruption of epigenetic marks (DNA methylation). *Aims.* We have studied the methylation status of the differentially methylated region (DMR) of GTL2 promoter in order to detect epigenetic alterations of DLK1/GTL2 gene. *Methods.* We have studied 8 patients, 6 males and 2 females, with myelodysplastic syndromes (MDS) classificated according to the FAB system; 2 patient with RA (25%), 3 with RAEB (37.5%), 2 with RAEB-T (25%), and 1 with CMML (12.5%). Median age was 68.4 years (range 38-88). Cytogenetic analysis: 2 patients had complex karyotypes (more than 3 cytogenetic abnormalities), 4 patients had apparently normal karyotypes, 1 had 45,XX,-7 karyotype , and 1 had 47, XX,+8 karyotype . None of the patients had ever received therapy with hypomethylating agents. DNA methylation pattern was determined by methylation-specific PCR of samples previously subjected to bisulphite-treatment, according to preestablished procedures. Subjects who have undergone bone marrow aspiration for diagnosis of thrombocytopenia, and after we had excluded hematological malignancies, served as controls. *Results.* We have studied the methylation pattern in both blood and bone marrow. The normal pattern consists of 2 bands (alleles), namely one corresponding to the methylated paternal allele, (size 160 bp) and one corresponding to the unmethylated maternal allele (size 120bp). We have found that alterations of the DMR were present in 4 (50%) of the patients studied: 2 (25%) had an abnormal methylation pattern in both blood and bone marrow samples and 2 (25%) others presented the same abnormal methylation pattern only in blood samples. No alteration of the methylation pattern was observed in the remaining 6 bone marrow samples. In the remaining 4 samples only the methylated allele was present. *Summary/Conclusions.* It is known that DLK1 gene is overexpressed in patients with MDS. A total of 16 samples were studied and 6 (37.5%) were found to be abnormal. It is probable that LOI through epigenetic modifications in the DMR of the GTL2 gene represents a potential pathogenetic mechanism in MDS. These are preliminary results and the study is ongoing. We are going to analyze DNA from a larger number of patients in order to verify our preliminary findings and to study further the imprinting status of the gene.

1346

COMPARISON OF IN VITRO GROWTH OF GRANULOCYTE-MACROPHAGE COLONY (CFU-GM) FORMATION IN PATIENTS WITH MYELODYSPLASTIC/MYELOPROLIFERATIVE DISEASES (MDS/MPD), TYPICAL MYELODYSPLASTIC SYNDROMES (MDS) AND MYELOPROLIFERATIVE DISORDERS (MPD)

E. Wiater, J. Dwilewicz-Trojaczek, G. Charlinski

Medical University, WARSAW, Poland

Myelodysplastic/myeloproliferative diseases (MDS/MPD) are a new category of disorders, which is separated by WHO classification. This group consist of 4 type of disorders: Chronic Myelomonocytic Leukaemia (CMML), atypical Chronic Myelogenus Leukaemia (aCML), Juvenile Myelomonocytic Leukaemia (JMML) and Chronic Myelodysplastic/Myeloproliterative disease-unclassifiable (MDS/MPD-U). We still lack understanding of disturbances of hematopoiesis in patients (pts) with MDS/MPD. The objective of the present study was to examine of hematopoiesis in pts with MDS/MPD compared pts with typical MDS and MPD *in vitro*. We enrolled 96 pts: 59 pts with MDS (RA-4, RARS-2, RCMD-17, RCMD/RS-7, 5q(-)-5, RAEB1-14, RAEB2-7, MDS-U-3), 12 pts with MPD (CML-5, OMF-4, CMPD-2) and 25 pts with MDS/MPD (aCML-6, CMML1-8, CMML2-6, MDS/MPD-U-2). Human CFU-GM cells were cultured by plating 1 x 105 mononuclear cells to semisolid methylcellose medium without or with cytokines (GM-CSF or G-CSF or SCF+GM-CSF+IL-3+Epo). CFU-GM colonies were scored at day 14. We compared spontaneus growth of CFU-GM and in presence of cytokines between patients with MDS/MPD, MDS and MPD. All of the results have been statistically tested by using T-student test for the independent groups. For statistically significant results were p<0,05. In pts with MDS/MPD according to pts with typical MDS: the spontaneus growth (respectively: med. 2 vs 0; p=0.0042), the growth with G-CSF (respectively: med. 13 vs 3; p=0.0016) and the growth with GM-CSF (respectively: med. 83 vs 16.5; p=0.042) of CFU-GM were statistically significant higher. In pts with MDS/MPD according to pts with MPD: the spontaneus growth (respectively: med. 2 vs 76; p=0.0003), the growth with G-CSF (respectively: med. 13 vs 146; p=0.0042), with GM-CSF (respectively: med. 83 vs 319; p=0.010) and with GM-CSF+IL-3+Epo (respectively: med. 68 vs 224; p=0.010) of CFU-GM were statistically significant lower. The growth of CFU-GM in pts with CMML1 was statistically significant lower according to pts with CMML2 in culture with G-CSF (respectively: med. 5 vs 184.5; p=0.031) and with GM-CSF (respectively: med. 17.5 vs 341.5; p=0.023). Statistically significant differences in culture of CFU-GM between pts with: MDS/MPD, typical MDS and MPD verify distinct biology of MDS/MPD. Statistically sig-

nificant differences in growth of CFU-GM in culture with G-CSF and with GM-CSF between pts with CMML1 and CMML2 show another biology isolated by WHO classification subtypes of CMML.

1347

BLAST CELL COUNT IN THE BONE MARROW OF PATIENTS WITH MYELODYSPLASTIC SYNDROME (MDS) OR SECONDARY ACUTE MYELOID LEUKEMIA (SAML): COMPARISON BETWEEN MORPHOLOGIC ASSESSMENT ON MARROW ASPIRATE (AS) AND IMMUNOHISTOCHEMISTRY ON BONE MARROW BIOPSY (BMB)

M. Tassara, M. Ponzoni, L. Camba, P. Ronchi, A. Crotta, J. Peccatori, C. Doglioni, F. Ciceri, M. Bernardi

San Raffaele Scientific Institute, MILAN, Italy

Backgrounds. in the French-American-British (FAB) co-operative group and the WHO classification MDS are stratified accordingly to marrow blasts percentage. Blast cell count is also comprised within the international prognostic score system (IPSS), which enables to define four groups with distinct prognosis. Diagnosis of sAML is defined by marrow blast count >30% (FAB) or >20% (WHO), along with previous diagnosis of MDS or dysplastic features of marrow myeloid lineages. Accordingly, precise quantitation of marrow blasts is critical both for diagnosis and prognosis of pts with MDS. AS is currently retained the best tool to assess hematopoietic cellular morphology; actually, quantitation of blasts is afforded by BMB when AS is not availabile (e.g. dry tap). *Aim*: to compare marrow blasts percentage quantified by morphology alone on AS and CD34+ blasts by immunohistochemistry on BMB in MDS and sAML patients. *Methods.* we reviewed the marrow aspirate and core biopsy reports of 169 pts with MDS or sAML at diagnosis, period 1997-2005. Marrow blasts have been morphologically quantified on May-Grunwald Giemsa stained AS and expressed as blast percentage over 500 nucleated marrow cells. Bouin's fixed, paraffin-embedded BMB have been evaluated for CD34+ immature cells counted over 1000 nucleated marrow cells. Diagnoses (FAB) according to morphology of marrow aspirate were: RA=106, RAEB=34, RAEB-T=12, CMML=5, sAML=12. According to FAB, WHO and IPSS, marrow blasts percentages have been grouped into four classes: A=0-4, B=5-9, C=10-19, D_20. Each marrow AS and BMB from single patient has been assigned a class and compared. *Results.* 50 cases (29.6%) showed a difference in blasts percentage, thus determining a class discordance; details are reported in the table. Most importantly a difference >1 class was observed in 10 cases (5.9%); in 5 cases blast cell count was higher and in the remaining 5 lower on BMB when compared to AS. *Conclusions.* this large retrospective mono-institutional study highlights the necessity to perform blast cell count both in AS and BMB of patients with suspected MDS/sAML at diagnosis. Immunohistochemistry for CD34 on BMB is useful in those cases with low (< 10%) blastic marrow infiltration. Major differences in blast counts (difference >1 class) have been evidenced only in a strict minority of evaluated cases, thus allowing a reliable blast cell count also on BMB.

Aspirate	*Biopsy*	*N°*
A	B	22
A	C	4
A	D	0
B	A	2
B	C	3
B	D	1
C	A	5
C	B	4
C	D	7
D	A	0
D	B	0
D	C	2
TOT		50

1348

BCR-ABL/ABL RATIO AND FISH PH POSITIVITY - RELATION TO C-KIT EXPRESSION AND DUAL ESTERASE ACTIVITY IN CML PATIENTS ON GLEEVEC THERAPY

M.R.A. Radic Antolic, S.M. Sucic, Z.R. Zadro, D.M.S. Davidovic-Mrsic, M.G.M. Markovic-Glamocak, R.S. Ries, G.K.K. Gjadrov-Kuvezdic, V.LJ. Vrbanus, B.D. Batinic, D.K. Dubravcic, J.M. Juricevic, L.B. Labar

Clinical Hospital Center Zagreb, ZAGREB, Croatia

Backgrounds. Chronic myelogenous leukemia (CML) is a myeloproliferative disease with t(9;22) and/or BCR/ABL fusion gene. One of treatment options for CML patients is inhibition of BCR/ABL and c-kit (CD117) tyrosine kinase activity with imatinib mesylate (Gleevec). *Aim of the study*: To analyze CD117 expression and dual esterase activity in bone marrow hematopoietic cells (HCs) of CML patients on Gleevec therapy and to correlate the results with percentage of FISH Ph positive HCs and bcr-abl/abl ratio. *Methods.* 22 bone marrow specimens of 17 CML patients on Gleveec therapy (duration of therapy 4 - 32 months) were analyzed by FISH and quantitative RT-PCR, immunocytochemical APAAP CD117 expression and cytochemical dual esterase activity. Patients were divided in subgroups according to duration of therapy (less than 6, 6-12 and more than 12 months). *Results.* Patients with leukocyte differential count changes had high bcr-abl/abl ratio, FISH Ph and CD117 positive HCs. Medians of CD117 and FISH Ph positive HCs were highest in CML patients during the first 6 months of Gleveec treatment, but correlation of these two parameters was low (0.19). There was no statistical difference when medians of CD117 and dual esterase positive HCs were compared between subgroups. Correlation of FISH Ph positive HCs and bcr-abl/abl ratio was high (0.68) with constant decrease in percentage of FISH Ph positive HCs and bcr-abl/abl ratio during follow-up. *Conclusions.* According to low correlation obtained between CD117 expression and dual esterase activity with percentage of FISH Ph positive HCs and bcr-abl/abl ratio, FISH and quantitative RT-PCR are the methods of choice for monitoring the efficiency of Gleevec therapy.

1349

THE EFFECT OF HYPERTHERMIA ON DIFFERENTIATION INDUCTION AND APOPTOSIS OF K562 ERYTHROLEUKEMIC CELL LINE: RELATIONSHIP WITH HEAT SHOCK PROTEIN 70 (HSP70)

L.S. Sharifkhatibi,[1] A. Kariminia,[2] N. Heidari,[1] S. Khoei,[1] B. Goliaei[1]

[1]Institute of Biochemistry and Biophysics, TEHRAN, Iran; [2]Pasteur Institute of Iran, TEHRAN, Iran

Hyperthermia causes a variety of morphological and functional effects in various cancer cells. At mild temperatures, hyperthermia can induce differentiation in different tumor cells including leukemia cells, but severe treatments cause cell death by apoptosis or necrosis. Hyperthermia also effects on heat shock protein gene expression. Since heat shock protein 70 (HSP70) has a crucial role in cell differentiation and cytoprotection, this protein may have a rule in differentiation and apoptosis induced by hyperthermia in K562 erythroleukemia cells. In the present work we have studied the effects of mild and severe treatments on differentiation induction and apoptosis in K562 cells. For this purpose, differentiation and apoptosis were measured along with the level of HSP70 protein. Erythroid differentiation was measured by benzidine staining assay and analyzing the expression of glycophorin A by flow cytometry technique. Apoptosis was evaluated by flow cytometric method based on binding of AnnexinV and DNA staining by PI. DNA fragmentation was also studied. HSP70 protein level was determined by HSP70 ELIZA kit. Our results showed that mild hyperthermia (43oC) reduced cell growth and induced differentiation without affecting cell viability but heating cells at 45°C reduced the viability and totally inhibited the growth of theses cells and no sign of differentiation was observed. On the other hand, mild hyperthermia (43°C) had not significant effect on induction of apoptosis in these cells, while, 45oC temperature caused cell death by apoptosis and necrosis. The level of HSP70 protein increased in cells treated with 43°C compared to the control cells, while, no significant increase could be detected at 45°C. In conclusion, increase in HSP70 protein level in 43°C heated cells can cause cytoprotection and lead to their differentiation, while, severe treatment, which cause no increase in HSP70 protein level, may lead to apoptosis of these cells.

1350

MUCOSITIS IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML) ON IMATINIB, AN INDIAN EXPERIENCE

A. Venkata Satya Suresh, V. Kamal, G. Anupama, P. Bapsy, C.J. Devaraj

Kidwai Memorial Institute of Oncology, BANGALORE, India

To evaluate the incidence and severity of mucositis in patients with chronic myeloid leukemia (CML) on Imatinib. Retrospective data analysis conducted at Kidwai Memorial Institute of Oncology, Bangalore, India, a tertiary care cancer center with an annual attendance of 16000 new cases. All patients of CML who were on Imatinib were analysed. They were stratified into chronic phase (CP), accelerated phase (AP), and blast crisis (BC). The CTC criteria was used to assess mucositis. A total of 210 patients with complete clinical data were analysed. Details are shown in Table 1.

	CP	AP	BC	P
Total patients	175	25	10	0.01
M:F Ratio	1.3:1	1.5:1	1:1	Na
Mucositis – total number	45	25	10	<0.01
Grade 2	28	8	5	NA
Grade 3	17	7	4	NA
Developing within 3 months	40	12	8	
Developing after 3 months	5	3	1	<0.01
Dose of Imatinib	400	600	800	

The majority of patients (90%) in BC developed mucositis, while AP (60%), and CP (26%) had a lower incidence. Mucositis onset was within the first 3 months of initiating Imatinib in the majority (87%) of the patients (p value <0.01). however, no patient required dose reduction or cessation of therapy due to mucositis. The median time for resolution of mucositis was 6 weeks irrespective of the stage of CML.

1351

IDENTIFICATION OF A RARE E6A2 BCR-ABL FUSION TRANSCRIPT IN CHRONIC MYELOID LEUKEMIA: A CASE REPORT

E. Di Bona,[1] N. Guercini,[2] I. Giaretta,[1] M. Frezzato,[1] M.C. Miggiano,[1] A. D'Emilio,[1] E. Albiero,[1] D. Madeo,[1] F. Rodeghiero[1]

[1]San Bortolo Hospital, VICENZA, Italy; [2]Center Human Genetics S.Bortolo Hospital, Vicenza, Italy

The balanced translocation t(9;22)(q34;q11) producing the chimeric BCR-ABL transcript is the hallmark of chronic myeloid leukemia (CML). Commonly, the breakpoints in the BCR gene occur within the major breakpoint cluster region (M-bcr), producing different types of BCR-ABL transcripts (e13a2 and/or e14a2). Breakpoints outside M-bcr occur rarely, in either minor-bcr (m-bcr) or micro-bcr (μ-bcr) leading to e1a2 and e19a2 fusion transcripts, respectively. Atypical BCR breakpoints outside these cluster regions are very rare. In particular, five cases of Philadelphia (Ph) positive CML and one case of CMML in progression have been associated with an atypical e6a2 BCR-ABL fusion transcript. The breakpoint in bcr intron 6 has been implicated as the cause of a more aggressive clinical phenotype and of increased oncogenic potential, due to the partial loss of important regulatory BCR sequences. Clinical outcome of CML expressing e6a2 fusion transcript and imatinib efficacy are not well established. AIM: We describe clinical and molecular features of a new case with Ph+ CML expressing e6a2 fusion transcript. CASE REPORT: A 54-year old man was admitted to Surgery Unit because of occlusive arteriopaty of the legs. His peripheral blood count showed mild anaemia (hemoglobin 10,7g/dL), thrombocytopenia (platelets 111×10^9/L), and leukocytosis (WBC $24,5\times10^9$/L, with 30% neutrophils, 41% lymphocytes, 7% monocytes, 17% eosinophils and 5% basophils). Ten days later the patient was referred to our Haematology Department, because of persistent leukocytosis and eosinophilia with hepatosplenomegaly. Microbiological and serological tests were negatives for viruses and parasites. Bone marrow examination showed high cellularity with hypereosinophilia and 10% blast cells. The presence of BCR-ABL and CBFB-MYH11 fusion genes was investigated by reverse transcription polymerase chain reaction (RT-PCR). Negativity for CBFB-MYH11 was demonstrated whereas an atypical amplification product larger than e1a2 fusion transcript was obtained with primers designed to detect breakpoint in m-bcr. Direct sequencing of the PCR product demonstrated a fusion between exon e6 of bcr gene and exon a2 of abl gene, resulting in an e6a2 BCR-ABL transcript. Cytogenetic analysis demonstrated 46,XY,t(9;22)(q34;q11)(100%) karyotype with no additional chromosomal abnormalities. Accelerated phase of CML was diagnosed. The patient was treated with imatinib with initial good haematological control of eosinophilia; however, 4 months later, cytogenetic analysis showed a karyotype evolution [46,XY(20%)/46,XY,t(9;22)(q34;q11)(10%)/ 47,XY,t(9;22)(q34;q11),+8(70%)]. Clinical and haematological features of progressive disease became evident after 7 months of treatment with imatinib; at this time cytogenetic analysis confirmed the selective advantage of the cellular clone with trisomy 8 [46,XY(5%)/46,XY,t(9;22)(q34;q11)(5%) /47,XY,t(9;22)(q34;q11),+8(90%)]. Myeloid blast crisis was diagnosed 11 months after first observation. The patient resulted primary resistant to chemotherapy based on idarubicin, etoposide and cytarabine. Because of severe pulmonary infection, no others treatments were given and the patient died of progressive disease. Conclusions. Presence of atypical e6a2 transcript in this case seems to indicate a poor prognosis. In particular, imatinib therapy was ineffective, in contrast to 3 recently reported cases. Ongoing mutation analysis is required to assess if the lack of response to imatinib in our case is due to e6a2 transcript in itself or to additional critical mutations in the ABL kinase domain.

1352

CCN3 EXPRESSION MAY PROVIDE AN ADDITIONAL MARKER OF RESPONSE TO IMATINIB

L. McCallum,[1] W. Lu,[1] R. Frazer,[1] S. Price,[1] M.F. McMullin,[2] R. Cuthbert,[2] A.E. Irvine[1]

[1]Queen's University Belfast, BELFAST, United Kingdom; [2]Belfast City Hospital, BELFAST, United Kingdom

Chronic Myeloid Leukemia (CML) is characterized by expression of the constitutively active Bcr-Abl tyrosine kinase. Molecular monitoring of BCR-ABL expression levels by Real-time PCR allows profiling of minimal residual disease for patients being treated with imatinib. We have shown previously in cell line models that the negative growth regulator, CCN3, is downregulated as a result of Bcr-Abl kinase activity and that CCN3 has a reciprocal relationship of expression with BCR-ABL. The aim of this study was to examine the relationship between BCR-ABL and CCN3 in primary human cells and determine if CCN3 expression could provide an additional marker of molecular response. Real-time PCR was used to determine CCN3 and BCR-ABL expression in CML patients undergoing treatment with imatinib and in normal donors. Gene expression was normalised against ABL as a control gene. Gene expression is reported as a percentage relative to ABL expression per 5 μL cDNA reaction. Western blotting and confocal microscopy was used to examine CCN3 protein levels in normal bone marrow samples and bone marrow from CML patients at diagnosis and following imatinib treatment. BCR-ABL burden in CML bone marrow (BM) samples at diagnosis was high (median 238.5%, n=11). In contrast CCN3 expression in these samples was low (median 0.9%). Patients with a complete cytogenetic response (CCR) and approaching molecular remission (less than 10 BCR-ABL transcripts (median 0.6%)) in follow-up peripheral blood (PB) showed a significant increase in CCN3 expression approaching that observed for normal PB. The median CCN3 increase was16.3% for CML patients responding to imatinib (p=0.005) and the median follow-up period was 28 months (range 3-60); median CCN3 expression for normal PB is 30.13%. Western blotting showed high CCN3 protein expression in normal BM (n=3). CCN3 expression was weak or absent in 3 CML BM samples at diagnosis and returned to levels comparable with normal BM upon response to treatment. Similarly, CD34+ cells extracted from CML BM showed increasing CCN3 expression with imatinib treatment (1 micromolar for 72h) in vitro (median increase 47%, n=3). Confocal microscopy showed only occasional weak staining in mononuclear cells from CML patients at diagnosis. Upon entering complete cytogenetic remission, the majority of cells stained positively for CCN3 expression. CCN3 expression has a reciprocal relationship with BCR-ABL in CML cell lines and primary human CML cells. Imatinib treatment of CML cells increases CCN3 expression. CCN3 expression may prove to be a useful marker in monitoring patient response to imatinib.

1353

INDUCTION OF DIFFERENTIATION AND APOPTOSIS IN NB4 LEUKEMIA CELLS BY A NOVEL DITEREPENE ESTER, 3-HK

M.A. Moosavi,[1] R. Yazdanparast[2]

[1]Institute of Biochemistry and Biophysics, TEHRAN, Iran; [2]Institure of Biochemistry and Biophysics, TEHRAN, Iran

Backgrounds. 3-hydrogenkwadaphnin (3-HK) is a new diterpene ester, recently isolated from the leaves of Dendrostellera lessertii with apoptotic, differentiating and anti-metastatic activities. Recently, we reported that the drug is very effective against leukemia cell lines without any detectable effects on normal cells. *Aim.* The aim of study is whether 3-HK can induce differentiation and apoptosis in a human APL cell line, NB4. *Methods.* cell cycle analyses by flow cytometry, apoptosis was assayed using annexin-V, DNA fragmentation, and Hochest staining. Differentiation was performed using Wright-Giemsa staining, NBT reduction and phagocytic activity. *Result.* The drug between 24 to 96 h

induced 7-65% growth inhibition of NB4 cells. Cell viability was also decreased by 2-55% between 24 to 96 h treatments with the drug. These effects of the drug were also dose-dependent. 3-HK induced a significant G1-arrest up to 48 h which consequently followed with appearance of sub-G1 peak (apoptosis) at 72 and 96 h. In addition we confirmed that the inhibition of proliferation is associated with differentiation especially toward macrophage-like morphology. *Conclusion*. We showed that 3-HK is a potent differentiating and apoptotic agents. These results can introduce 3-HK as potent candidate to treatment of leukemia.

1354

THE ROLE OF MDR RELATED PROTEINS IN THE PROGNOSIS OF ADULT ACUTE MYELOID LEUKEMIA (AML) WITH NORMAL KARYOTYPE

M. Tiribelli,[1] D. Damiani,[1] D. Raspadori,[2] A. Michelutti[1], A. Gozzetti,[2] E. Calistri,[1] A. Candoni,[1] A. Chiarvesio,[1] M.P. Lenoci,[2] D. Russo,[3] R. Fanin[1]

[1]*Division of Hematology and BMT, UDINE, Italy;* [2]*Chair and Division of Hematology, SIENA, Italy;* [3]*Chair of Hematology, Unit of B.D./C.T., BRESCIA, Italy*

Background. Cytogenetic abnormalities are among the most important factors affecting the outcome of patients with acute myeloid leukemia (AML), but approximately 40-50% of AML cases display a normal karyotype at diagnosis. In the last years the over-expression of MDR related proteins has emerged as a factor negatively affecting outcome in leukemia patients, especially in cases with abnormal karyotype. Less defined is the impact of drug-transporter proteins in AML with normal diploid cytogenetics. *Aims*. We have compared the expression of P-glycoprotein (PGP), multidrug-resistance related protein (MRP) and lung resistance protein (LRP) with the clinical and biological characteristics of 135 adult patients with normal karyotype AML, to evaluate their possible impact on response to therapy and on survival. *Methods*. Median age was 53 years and 60 out of 135 (44%) patients were older than 55 years. Therapy consisted of a standard 3/7 (idarubicin and cytarabine) course as induction and intermediate-dose cytarabine (2 courses) as consolidation for 38 patients. Fludarabine-based induction course (FLAI / FLAIE) followed by intermediate-dose cytarabine as consolidation was used in 65 patients. Thirty-two patients were included in a clinical trial comparing FLAI to ICE as induction course, followed by a consolidation course of high-dose cytarabine. For statistical analysis response to therapy was evaluated after two chemotherapy courses. Patients who underwent allogeneic stem cell transplantation were censored at time of transplant. *Results*. Increased PGP expression was associated only to advanced age (p=0.003). Conversely, no difference in the two age cohorts was found in MRP and LRP expression. No association was assessed between PGP, MRP and LRP over-expression and clinical and biological characteristics. Complete remission was strongly affected by PGP over-expression. In fact only 13/84 (15%) PGP-negative, but 19/43 (44%) PGP-positive patients did not respond to chemotherapy (P = 0.006). Advanced age and CD34 positivity on blast cells confirmed their negative role on obtainment of remission. No impact on response to therapy was demonstrated for MRP and LRP. However, a lower percentage of complete responses was observed in those patients over-expressing more than one MDR protein (p=0.03). Event-free survival of the whole population was 9 months. In the univariate analysis EFS was influenced by PGP over-expression (10 vs 4 months, p=0.035). EFS was negatively affected also by age (p=0.0008) and CD56 (p=0.044). In multivariate analysis all this factors retained their statistical significance. *Summary/Conclusions*. In our study only PGP expression showed a negative correlation with response to induction therapy, as well on EFS. MRP or LRP did not influence treatment outcome when singularly considered, but patients over-expressing more than one MDR-related protein had a lower probability to achieve CR. Age was the most important factor affecting EFS, but shorter EFS duration was observed also in PGP-positive patients and in those with CD56 aberrant expression. Our data confirmed the prognostic role of MDR proteins also in the subset of AML patients with normal karyotype, and could be used to stratify patients with different prognosis and to design risk-adapted therapeutic strategies.

1355

THE EVALUATION OF ANTI TUMORAL AND DIFFERENTIATION OF PLANT-DERIVED AGENTS IN COMBINATION WITH ATRA ON LEUKEMIC CELLS

F. Zaker,[1] A. Oody,[1] M. Soleimani[2]

[1]*Iran University of Medical Sciences, TEHRAN, Iran;* [2]*Tarbiat Modaress University, TEHRAN, Iran*

Backgrounds. Acute leukaemia is characterised by accumulation of neoplastic cells which fail to develope into mature cells. Cytotoxic, differentiation and apoptotic agents have been employed for treatment of leukaemia. In iranian traditional medicine plant-derived agents have been used for treatment of cancer. *Aims.* The present study is evaluation of cytotoxcicity, apoptosis and differentiation of several plant extracts such as Peganum Harmala, Harmine, Harmaline, Urtica Dioica, Chelidonium Majus and Viscum Album on HL60 cells . ATRA has been used as standard agent. However, little study have been reported using these agents on this cell line, these components were initiated such as an investigation. *Methods.* HL60 cells were cultured and plant extracts added to cells and incubated for 5 days. Counting of cells, viability, MTT, morphology, NBT reduction and cytofluorometric analysis performed by FACS using PI for cell cycle ,markers including CD11b and CD14 for myeloid differentiation and apoptosis using Annexin V. *Results.* The data showed that all agents in optimal dose caused cessation of proliferation in dose and time dependent manner(p<0.05). Optimal concentration of Peganum Harmala, Harmine, Harmaline, Urtica Dioica, Chelidonium Majus and Viscum Album (10 μg/ml,1.6 μg/ml,10 μg/mL, 2.5 mg/mL ,0.1 mg/mL and 50 μg/mL respectively) were chosen as antiproliferative effect with good viability. However, all agents in higher concentration were cytotoxic. Treated cells with ATRA showed depletion of growth in optimal dose of 10-7 Mol. Cells accomulated in G1 phase using ATRA (81.5%), Urtica Dioica (75%) and Viscum Album (72%) but they arrested in S phase using Peganum Harmala, Harmine, Harmaline (52.7%) and Chelidonium Majus (54.5%). Only, cells induced by Harmaline 10 micg/ml showed myeloid differentiation with some morphological changes , NBT positivity (28%) and increase in CD11b (24.3%) and CD14 (43.5%) (p<0.05) compared to ATRA (40% as NBT, 71% and 5.7% as CD11b and CD14).However, Viscum Album showed some apoptotic changes in 100 micg/ml concentration.The combination of these agents in optimal dose with ATRA did not show any effect on differentiation of cells and ATRA preserved effect of differentiation of itself with higher cessation of proliferation. *Conclusions.* In conclusion , these data showed that the combination of these plant extracts with cytotoxic and differentiation agents may open a new window in leukemic in vitro therapy which requires further investigation.

1356

NO INFLUENCE OF A POLYMORPHISM IN ENDOSTATIN, AN ANGIOGENESIS INHIBITOR, FOR THE RISK OF ACUTE MYELOID LEUKEMIA

C. Lima, H. Nascimento, G. Loureno, G. Yamaguti, F. Costa

State University of Campinas, CAMPINAS, Brazil

Backgrounds. Angiogenesis is an important step in solid tumours and leukaemias development and progression. In addition to producing proangiogenesis cytokines, there is evidence that neoplastic cells also play a role in the generation of endogenous antiangiogenic proteins, such as endostatin (ES). ES is a 20kDa C-terminal fragment of collagen XVIII, the product of the COL18A1 gene. Higher serum levels of ES induced experimentally in mice caused regression of leukaemia and solid tumours. In addition, Down's syndrome patients have a decreased incidence of solid tumours possibly due to the high serum levels of the protein produced by their three copies of the COL18A1 gene. Thus, different levels of ES seem to be associated with varying susceptibility to tumour development. Furthermore, a COL18A1 gene polymorphism (D104N) located in the COOH-terminal globular domain, NC1, of collagen XVIII, the encoding region for ES was recently associated with increased risk for the prostatic adenocarcinoma, which was attributed to an impairment in the protein function. Aims. In this study, we tested whether D104N polymorphism of the COL18A1 gene alters the risk for AML. Methods. Genomic DNA from peripheral blood of 122 AML patients (74 men, 48 women; mean age±SD: 46.8±17.9 years; 106 Caucasian, 16 Blacks), seen at the University Hospital of the State University of Campinas, and 351 controls (198 men, 153 women; mean age±SD: 52.9±4.5 years; 302 Caucasians, 49 Blacks) were analysed using the polymerase chain reaction (PCR) followed by restriction endonuclease digestion with Mse I. Results. Both the patients' and controls' samples were in Hardy-Weinberg equilibrium (X^2 = 0.77, p= 0.379; X^2 = 1.89, p=0.17,

for heterozygous D104N genotypes, respectively). We have observed similar frequencies of D104N genotypes in AML patients and controls (14.8% and 13.7%, respectively; p=0.76). Similar risks for the disease were also seen in individuals with heterozygous D104N polymorphism in comparison with the wild genotype (OR= 1.09, 95%CI: 0.61-1.96). Considering only the AML patients, no differences in the frequencies of D104N polymorphism were found according to gender (12.2% in male vs 18.8% in female; p=0.44), age (9.6% in 73 patients under 50 years vs 22.5% in 49 patients at an older age; p = 0.07), and ethnic origin (15.1% in Caucasian patients vs. 12.5% in Black patients; p = 1.00). Conclusion: Our results present preliminary evidence that the D104N polymorphism of the COL18A1 gene may be an unimportant determinant of the AML susceptibility.

Funding: Supported by FAPESP.

1357

OUTCOME OF ALLOGENEIC STEM CELL TRANSPLANTATION IN ADULT PATIENTS WITH PH+ AML

H.J. Kim, W.S. Min, K.S. Eom, Y.J. Kim, S. Lee, C.K. Min, D.W. Kim, J.W. Lee, C.C. Kim

CHSCTC,Catholic Univ. of Korea, SEOUL, South-Korea

Backgrounds. The Philadelphia chromosome positive (Ph+) acute myelogenous leukemia (AML) is rarely found in adult patients with an overall incidence of less than 1%. Most patients with Ph+ AML have an extremely poor prognosis when treated by chemotherapy alone. Recently, allogeneic hematopoietic stem cell transplantation (HSCT) performed early during remission with an improved treatment agent, using an interim therapy of imatinib (Glivec, STI571), has suggested a long-term survival. *Aims.* To better understand a role of imatinib in adult Ph+ AML and envision the future treatment strategy, we analyzed the effect of imatinib addition into standard chemotherapy as an alternative before allogeneic HSCT for newly diagnosed Ph+ AML. *Methods.* Between Nov 2001 and Oct 2004, 12 (2.2%) of the 556 adults (age, 17~84) with AML were Ph+ at the time of diagnosis. Of these, 5 patients with newly diagnosed Ph+ AML who completed induction chemotherapy and received matched or mismatched allogeneic HSCT were investigated in this study. Overall complete remission rate was 58.3% (7/12) by intention-to-treat analysis. Two patients were excluded because of their refusal of further treatment. All the patients were treated according to our center's standard protocol, which consists of 5x10 idarubicin (IDA) plus N4-behenoyl-1-b-D-arabinofuranosyl cytosine (BH-AC) induction chemotherapy. Patients who achieved CR after induction chemotherapy were routinely assigned to receive 400 mg or 600 mg imatinib daily. Subsequently, patients in CR received consolidation chemotherapy consisting of '3x5' IDA plus BH-AC followed by a second imatinib cycle bridging the time to HSCT. The preparative regimen consisted of total body irradiation (1320 cGy) and Busulfex (3.2 mg/kg for 2 days) for patients in first CR. Graft-versus-host disease (GvHD) prophylaxis was attempted by administering cyclosporine or tacrolimus plus methotrexate. Stem cell source was bone marrow (n=3), peripheral blood (n=1), and both (n=1). *Results.* With a median follow-up duration of 13 months (range, 2~20), 1 patient died early due to severe thrombotic thrombocytopenic purpura and muti-organ failure. Another 1 patient died 7 months post-transplant due to pneumonia with sepsis. However, according to every 3 month-period monitoring of minimal-residual disease by using real-time PCR method, the other 3 patients have been in excellent condition and all of them showed undetectable level of their BCR-ABL/ABL ratios without addition of imatinib after HSCT. All of them showed grade II of acute GvHD and progressed to limited type of chronic GvHD, but they responded well to conventional treatment modality. *Conclusions.* In comparison to old control data, first-line imatinib interim therapy appears to provide a good quality of CR and a survival advantage for patients with Ph+ AML after allogeneic HSCT. Further long-term follow-up with large sample numbers is needed to validate the results of this study.

1358

AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR NORMAL KARYOTYPE ACUTE MYELOID LEUKEMIA PATIENTS WITH FLT3 ITD MUTATIONS IN FIRST COMPLETE REMISSION

L.C. Lim, G.F. How, L.T. Tan, S. Fook-Chong, Y.C. Linn, L.H. Lee

Singapore General Hospital, SINGAPORE, Singapore

Traditionally patients with acute myeloid leukemia (AML) and normal karyotype have been regarded as intermediate risk. High dose chemotherapy followed by autologous peripheral blood stem cell transplantation (auto-PBSCT) as post remission therapy for intermediate risk AML patients in first complete remission (CR) has been reported to have encouraging results. We adopted this approach at our instiution. In the 5-year period from 1999 to 2003, 17 patients who satisfied these criteria underwent auto-PBSCT. They comprised 12 male and 5 females with ages ranging from 15-66 years (median - 48years). The aim of our study is to retrospectively analyse the outcome of these patients in relation to presence or absence of FLT3 mutations. Testing for the internal tandem duplication (ITD) and Asp835 FLT3 mutations were performed on samples obtained at time of diagnosis. Genomic DNA samples were analysed by polymerase chain reaction (PCR) for ITD and Asp835 with nucleotide sequencing for confirmation where aberrant PCR products were identified. It is important to determine the status from samples at diagnosis as we found that FLT3 ITD positive cases at diagnosis could subsequently lose the mutation and become negative when the post remission marrow samples were tested. FLT3 ITD was detected in 8(47%) patients but none were found to possess the Asp835 mutation. Comparison between the ages and sex of the FLT3 ITD positive with negative patients showed no statistical differences. However, the FLT3 ITD positive cases had a statistically significant higher presenting white cell count (range 2,500/ul-238,000/uL, median - 89,000/uL) than the negative cases (1,500/uL-77,600/uL, median - 11,300/uL) with p-value of 0.011. The followup period for all 17 patients ranged from 6-45.5 months (median - 25months). No patients were lost to follow up. The 3-year survival was 55.6% for FLT3 ITD positive compared with 33.3% for the negative cases. Overall survival (OS) was thus significantly better for those without FLT3 ITD mutations (p=0.05, log rank test). By Kaplan-Meier curve, FLT3 positive patients had a higher relapse rate. However this trend towards shorter disease free survival (DFS) for the FLT3 ITD positive (37.5%) versus negative (55.6%) cases at 3 years could not be demonstrated to be statistically significant (p=0.1709), possibly due to small sample size. In summary, determination of status of FLT3 ITD mutations at diagnosis is important in risk stratification management of patients with normal karyotype AML in first CR. Unlike the experience of Yoshimoto et al who found that myeloablative chemotherapy supported by auto-PBSCT in such patients may overcome the poor prognostic implications of FLT3 mutations, we have not found this to be so. Since 2004, we have amended our approach to offer allogenic bone marrow transplantation upfront to FLT3 ITD positive AML patients in first CR where possible

1359

PROGNOSTIC SIGNIFICANCE OF FLT3 MUTATIONS IN DIFFERENT SUBTYPES OF ACUTE MYELOID LEUKEMIA

N. Tosic,[1] N. Colovic,[2] V. Djordjevic,[2] B. Petrucev,[1] S. Pavlovic[1], M. Colovic[2]

[1]IMGGE, BELGRADE, Serbia and Montenegro; [2]Institute of Hematology Clin Cen Serbia, BELGRADE, Serbia and Montenegro

Backgrounds. Fms-like tyrosine kinase 3 (FLT3) is a member of the class III receptor tyrosine kinase family along with KIT, FMS and platelet derived growth factor receptor. Wild type FLT3 is expressed at high levels on 70% to 100% of blasts in acute myeloid leukemia (AML). FLT3 gene alterations, internal tandem duplications (ITDs) and Aspartate835 (D835) mutations occur in 15%-30% in AML and may adversely affect clinical outcome. *Aims.* The aim of our study was to analyze the impact of FLT3 mutations in cohort of 113 newly diagnosed patients with AML on prognosis. *Methods.* Genomic DNA polymerase chain reaction (PCR) assay was performed to detect FLT3/ITDs located from exon 14 to exon 15 and we used PCR-restriction fragment length polymorphism (PCR-RFLP) for detection of D835 mutations in exon 20. *Results.* FLT3/ITD were detected in 20/113 patients (pts) (17.6%), D835 mutations in 4/113 (3.5%) and both type of mutations in 1 (0.8%) pt. In the study group of 113 pts according to FAB classification, FLT3 mutations were found in all subtypes except M1, M6 and M7 The distribution of FLT3 mutations was as follows: FLT3/ITD was detected in M0 6/12 (50%) pts, 4/22 (16.7%) in M2, 3/14 (21.42%) M3, 3/24 (12.50%) M4, in 8/19 M5 pts (40.3%). D835 mutation was found in 1 pt with M2 and M5 and 2 pts with M4 type of AML. Of 24 pts with FLT3 mutations a normal karyotype was found in 9 pts, 3 pts had translocation (15; 17), 2 pts had inv (16), one deletion of 19. Three pts had complex karyotype, and in 2 pts there were no mitoses on preparation. Treatment included induction chemotherapy with doxorubicin 50 mg/m^2 3 days and cytosine arabinoside 200 mg/m^2 in continuous infusion for 7 days. Consolidation therapy consisted of the same scheme or ADE combination. Complete remission in the whole cohort of patients was achieved in 62% and in

only 7/24 (29%) pts with FLT3 mutations (one patient with M0 and normal karyotype, one with M5a and also normal karyotype, 3 pts with M3 and translocation (15; 17), one patient with M2 and deletion 19, one with M4 and inv16). FLT3/ITD+ pts had significantly higher WBC count at diagnosis (WBC count for FLT3/ITD+ was 73.5×10^9/L and WBC count for FLT3/ITD- was 14.9×10^9/L, $p<0.05$). Median of overall survival of the whole group of pts was 11 months, and median of survival of pts with FLT3 mutations was 6 months. *Conclusion*: In contrast to other reports incidence of FLT3/ITD and D835 mutations are lower in our cohort although the study group was small and performed in a single Institution. With a median follow up of 46 months remission duration and overall survival were significantly shorter for patients with FLT3 mutations.

1360

MONITORING OF CARDIOTOXICITY DURING INDUCTION CHEMOTHERAPY CONTAINING IDARUBICIN IN ACUTE MYELOID LEUKEMIA WITH CIRCULATING MARKERS

J.M. Horacek, L. Jebavy, P. Zak, M. Tichy, R. Pudil, L. Slovacek

Faculty Hospital, Charles University, HRADEC KRALOVE, Czech Republic

Backgrounds. Cardiac toxicity is a well-known and serious complication of antitumorous treatment. Anthracyclines represent the greatest risk for development of cardiotoxicity. Recently, circulating markers of structural and functional myocardial damage have been gaining ground in cardiotoxicity diagnostics. *Aims.* Monitoring of cardiotoxicity during induction chemotherapy in acute myeloid leukemia (AML) patients and assessment of the potential for use of circulating markers in early diagnostics of cardiotoxicity. *Methods.* Fifteen consecutive adult patients with a newly diagnosed AML (9 male and 6 female, mean age 43.7±10.6 years) participated in the study. The patients received induction chemotherapy containing intermediate doses of cytarabine and idarubicin (IDA) 12 mg/m^2/day intravenously on day 1, 3 and 5 (in total 36 mg/m^2 = 1/4 of the maximum recommended cumulative dose). From circulating markers of myocardial damage we used a marker of cardiac dysfunction and failure - N-terminal pro brain natriuretic peptide (NT-proBNP), and two markers of myocardial necrosis (cardiospecific markers) - cardiac troponin T (cTnT) and creatine kinase MB (CK-MB mass). Serial measurements of plasma NT-proBNP concentrations were performed at the baseline, the day following each IDA infusion, after 14 days and after circa 1 month, i.e. before the next chemotherapy. Cardiospecific markers (cTnT, CK-MB mass) were measured at the baseline and after the last IDA infusion. *Results.* The mean baseline concentration of NT-proBNP in newly diagnosed AML patients was 129.7±59.6 pg/mL. The mean NT-proBNP concentration increased after the first IDA infusion to 307.3±171.4 pg/ml (p=0.02). In most of the patients, the second and the third IDA infusions were not associated with a further increase in the NT-proBNP value and values after 2 or 4 weeks were not significantly different from the baseline. However, in one of the patients the NT-proBNP values were increasing after each IDA infusion (after the last one 786.2 pg/ml) and within 14 days he developed congestive heart failure due to left ventricular diastolic dysfunction as assessed by echocardiography. At that time, the NT-proBNP value was 1184.0 pg/ml; after diuretics it decreased significantly. In all patients, plasma cTnT and CK-MB mass concentrations were within the reference interval at the baseline and after the induction chemotherapy. *Conclusions.* Our results show that induction chemotherapy in AML (IDA 36 mg/m^2 and intermediate doses of cytarabine): 1. does not cause detectable damage of cardiomyocyte structure, 2. is in all patients associated with acute neurohumoral activation (transient elevation of NT-proBNP) indicating acute subclinical cardiotoxicity, 3. may lead to congestive heart failure and NT-proBNP seems to be a promising early marker and predictor of this complication.

Funding: Supported by Research Project MZO 00179906.

1361

WT-1, BCL2 AND BAX EXPRESSION AND CLINICAL OUTCOME IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

M. Miglino, M. Balocco, N. Colombo, R. Varaldo, R. Grasso, M. Clavio, G. L. Michelis, S. Aquino, F. Olcese, G. Catania, A. Albarello, F. Ballerini, I. Pierri, L. Canepa, P. Canepa, M. Gobbi

Department of Hematology-Oncology, GENOVA, Italy

Background and aims. We prospectively evaluated the impact of WT-1, BCL2 and BAX expression on the clinical history of patients with non M3 AML and present here our preliminary results. *Patients and methods*. Forty patients have been included in the study. Complete molecular data are at the present available of 15 patients. Median age was 55 years (range 24-73); the cytogenetic evaluation at diagnosis (14 patients) disclosed a normal karyotype in 9 patients, a complex karyotype in 3 and other abnormalities in 2. All patients received as induction therapy fludarabine, Ara-C, idarubicin plus etoposide; 13 achieved CR (3 after second line therapy), 2 were refractory. All patients in CR received consolidation chemotherapy (13) or were submitted to autologous or allogeneic stem cell transplant. CR lasted a median of 14.5 months (range 3-48). Five patients have relapsed after a median of 8 months (range 3-17). At this moment 10 patients are alive and disease free in first or second CR. Median survival for the whole series of patients is 18 months (range 8-50. By real time PCR we studied the expression of WT-1, BCL2 and BAX on marrow samples collected at diagnosis, at evaluation of response to induction and every 6 months, in the follow up and tried to correlate molecular data with clinical outcome. *Results*. We classified patients in 3 groups according to the expression at diagnosis of WT-1 and BCL2. The 3 patients with absent or low expression of WT-1 and BCL2 at diagnosis maintain CR at 28, 30 and 48 months. Four out of the 5 patients with high expression of WT-1 or BCL2 at diagnosis achieved CR; 1 did not respond to induction therapy. Among the responsive patients 1 has died in CR after 17 months for transplant related complications, 2 have relapsed (at 11 and 14 months from diagnosis) and 1 mantains CR. Of the 3 patients with high expression of both WT-1 and BCL2 at diagnosis 1 did not respond to therapy, 2 achieved CR (in one patient lasted 7 months, in the second still ongoing at 12 months). Whereas the expression of BAX at diagnosis doesn't seem to correlate with outcome, the level of BCL2 expression may have a relevant prognostic value. The 3 patients mantaining the first CR at 28-48 months had low levels af BCL2 expression at diagnosis. On the contrary patients with high levels of BCL2 had a poor outcome: one did not respond to therapy, other two showed a chemorefractory relapse at 7 and 15 months from diagnosis. The longitudinal evaluation of BAX-BCL2 ratio may give information on the relapse risk, with the progressive reduction of this ratio indicating impending relapse. The increase in BCL2 and therefore the reduction of BAX-BCL2 ratio may precede of some months the increase of WT1. *Conclusion*. These preliminary results indicate that molecular evaluation at diagnosis of WT-1, BCL2 and BAX might have prognostic value and that prospective comparative evaluation of BAX-BCL2 ratio might predict relapse.

1362

PROGNOSTIC RELEVANCE OF SOLUBLE TPO LEVEL IN AML

S. Aref,[1] T. Al-Khodery,[1] M. El-Sherbiny[2]

[1]*Mansoura University, MANSOURA, Egypt;* [2]*National Cancer institute, CAIRO, Egypt*

Backgrounds. Thrombopoietin (TPO), the major growth factor for cells of the megakaryocytic lineage is removed from circulation by binding to c-mpl receptors present on platelets and megakaryocytes. Recently functioning c-mpl receptors was reported on the AML blast cells and its clinical impact on AML prognosis remains to be characterized. *Aim.* Is to determine the level of TPO in AML patients in order to characterize its clinical relevance. *Methods.* We assessed TPO levels by ELISA in 41 AML patients at diagnosis, after 28 days of induction chemotherapy and at AML remission. Follow up for the patients was done up to 24 months. *Results.* TPO levels was significantly higher at diagnosis as compared to normal controls ($p<0.01$). At 28 days after induction chemotherapy the TPO level continue to elevate and was significantly higher as compared to the diagnosis level ($p<0.01$) ,and then decline during remission reaching near the control level ($p>0.05$). The TPO levels was inversely correlated to the platelets counts (R=0.9 , $p<0.01$). TPO level at AML diagnosis was significantly lower in a group of patients who died during the follow up course(n= 25) and in patients resist induction chemotherapy (n=8) as compared to patients who survive and patients who respond to chemotherapy ($p<0.05$ for both).

1363

IMPACT OF ADDITIONAL CYTOGENETIC ABNORMALITIES ON REMISSION INDUCTION RATE,EVENT FREE AND OVERALL SURVIVAL IN 34 PATIENTS WITH NEWLY DIAGNOSED ACUTE PROMYELOCYTIC LEUKEMIA TREATED WITH APL93.TUNISIAN EXPERIENCE

R. Jeddi,[1] H. Benneji,[1] K. Kacem,[1] S. Hdiji,[2] R. Belakhal[1], L. Aissaoui,[1] H. Benabid,[1] Z. Belhadjali,[1] A. Saad,[3] B. Meddeb[1]

[1]*Aziza othmana hospital, TUNIS, Tunisia;* [2]*Hopital Hdi Chaker, SFAX, Tunisia;* [3]*Hopital Farhat Hached, SOUSSE, Tunisia*

Additional chromosomal abnormalities in acute promyelocytic

leukaemia (APL) are observed in around 30% of cases. Their presence seems to not have any impact on prognosis. The aim of our study is to analyse impact of additional cytogenetic abnormalities on complete remission rate (CRR), event free survival (EFS) and overall survival (OS) in 34 consecutives patients with APL and t(15,17) treated with APL93 protocol between 1998 and 2004. Median age was 28 yr (6-60yr). Median WBC was 3000/mm^3 (600-97000/mm^3). Informative karyotype was obtained in all patients, additional cytogenetics abnormalities were seen in 9 patients:26,47% (9/34).These abnormalities were:+8(4),add 9q(1),del 9q12;q31(1),i der17q(1),add15p11(1),add 5p15(1). For all patients CRR was 82%(28/34), failure of induction was due to 6 toxic deaths: sepsis (1), ATRA syndrome (2), SNC hemorrhage (2), and diabetes (1). EFS at 4 yr is 63, 47% and OS at 4 yr is 69, 72%. Outcome was similar between patients with t(15,17) alone and patients with additional cytogenetic abnormalities for CRR:84%(21/25) vs 77.7%(7/9) p=0.6,for EFS at 4 yr :62,02% vs 66,67% p=0.74,and for OS at 4 yr:64,81% vs 70,88% p=0.5. Our study does not find any significative impact of additional abnormalities despite a little advantage for EFS and OS for this group.

1364

HUMORAL IMMUNE RESPONSE AGAINST THE PRAME ANTIGEN IN PATIENTS WITH MYELOID LEUKEMIAS

Y. Finashutina,[1] M. Schmitt,[2] J. Greiner,[2] A.V. Misyurin[1]

[1]*National Research Center for Hematology, MOSCOW, Russian Federation;* [2]*University of Ulm, ULM, Germany*

Backgrounds. The PRAME (preferentially expressed antigen in melanoma) is expressed at high levels in various malignant tumors including hematopoietic malignancies, especially in acute myeloid and lymphoid leukemias (AML, ALL), multiple myeloma etc. It has no or weak expression in normal tissues making it a candidate for immunotherapy. PRAME can also elicit T-cell immune response in melanoma patients but there are no data concerning anti-PRAME immune response in leukemias. *Aims.* To detect specific immune response towards PRAME in patients with myeloid leukemias (AML, CML). *Methods.* Sera obtained from patients with myeloid leukemias were analyzed in enzyme-linked immunosorbent assay (ELISA) for detection anti-PRAME antibodies. *Results.* IgG PRAME antibodies were measured in 122 patients (25 AML, 97 CML) and 22 healthy volunteers. Immunoglobulin IgG PRAME antibodies were detected in 4 (16%) and 8 (8%), respectively, of 122 patients, whereas none of the healthy volunteers had IgG PRAME antibodies. In one of IgG PRAME 'positive AML samples the specific cytotoxic T-lymphocytes were founded by MHC-peptide tetramer staining with intracellular interferon-γ co-staining. *Summary*: The data demonstrate that spontaneous humoral immune responses against PRAME protein could be detected in the patients with PRAME-expressing hematopoietic malignancies.

1365

IS LEUKAPHERESIS ABLE TO IMPROVE SURVIVAL IN HYPERLEUKOCYTIC AML IF USED AS THE EARLY CYTOREDUCTION TREATMENT?

Z.K. Koristek, M. Navratil, J. Mayer, M. Doubek

Masaryk University Hospital, BRNO, Czech Republic

Background. The management of patients with AML presenting with hyperleukocytosis remains controversial. In spite of relatively high incidence of hyperleukocytic AML (7-15%) and its very high early mortality (>40%), there is no consensus in the initial treatment for a prompt leukoreduction. *Aims*. The aim of this retrospective non-randomized study was to compare early mortality and overall survival (OS) in patients with hyperleukocytic AML initially treated with either hydroxyurea (HU) alone or HU and leukapheresis. *Patients and Methods*. From 1998 to 2005, 40 patients were treated for hyperleukocytic AML (M0=2, M1=8, M2=7, M3=2, M4=16, M5=5) in our institution. Group A consisted of 20 patients with median age of 67 years (19-78) treated by HU only (50 mg/kg/day in 3 or 4 daily doses). Group B consisted also of 20 patients with median age of 53 years (19-72) treated with HU and cytoreduction leukaphereses. The intention of the cytoreduction treatment was to decrease WBC count to at least 50x10e9/l before administration of an induction chemotherapy to prevent complications from leukostasis and tumor-lysis syndrome. Leukaphereses were performed using COBE Spectra cell separator. *Results*. The early mortality was high according to the expectations: seven patients died within two weeks in group A, as well as in group B. The patients from the group B were generally in worse condition and 4 of them died within the first 48 hours for intracranial hemorrhage or respiratory failure (ARDS) because of leukostasis. The target cytoreduction in the group A was delayed compared to the group B, although the initial WBC count was lower (160×10^9/L vs. 200×10^9/L, means). In the group B, forty leukaphereses were performed in total, median 2 (1-4) per patient. Induction chemotherapies could started earlier in the group B compared to group A: on day 4 (median, range 2-12, median WBC count 30.2×10^9/L) and on day 8 (median, range 1-14, WBC 24.0×10^9/L), respectively. Thirty induction chemotherapies were administered in total, 14 in the group A and 16 in the group B. One patient from the group B refused chemotherapy and died of leukemia in 11 days. Complete remissions were reached in 15 patients, but only in 5 from the group A. OS was significantly longer in the leukapheresis group (p<0.05), however, we did not confirm improvement of the 2-week mortality. Median OS in the group A was 30 days and no patient survived more than 500 days. Median OS in the group B is 282 days and 6 patients are still alive from 2 to 5.7 years after the AML diagnosis. *Summary/conclusions*. Current published data do not define the impact of using leukapheresis for the cytoreduction before induction chemotherapy on survival of patient with hyperleukocytic AML. It was only proved that application of leukapheresis reduced 2-week mortality (*Giles et al. Leuk Lymphoma, 2001;42: 67-73*), but there is no evidence of its influence for improvement of survival. We can conclude that in our study a significant improvement of overall survival was reached when leukapheresis was combined with hydroxyurea treatment.

1366

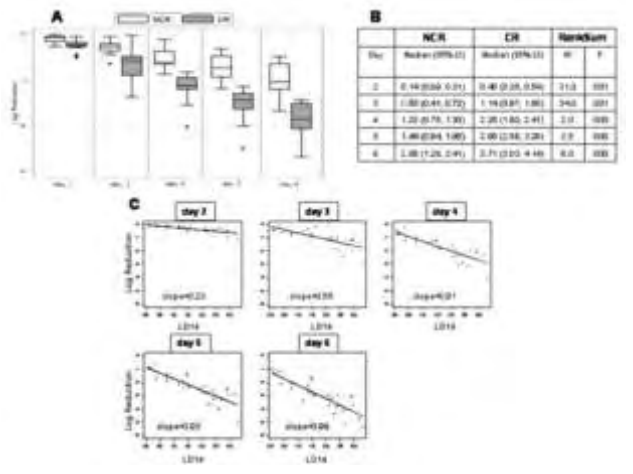
CLEARANCE OF LEUKAEMIC BLASTS FROM PERIPHERAL BLOOD DURING STANDARD INDUCTION TREATMENT PREDICTS THE BONE MARROW RESPONSE IN ACUTE MYELOID LEUKAEMIA: A PILOT STUDY

J. Voglova,[1] F. Mannelli,[2] M. Baccini,[2] E. Antonioli,[2] F. Leoni,[2] A. Bosi[2]

[1]*Department of Clin.Hematology, CHARLES UNIV. HOSPITAL, HRADEC KRALOVE, Czech Republic;*[2]*Azienda Ospedaliera Universitaria Careggi, FLORENCE, Italy*

Background. Although several parameters (i.e. age, cytogenetics and secondariness) are useful for risk stratification of patients with acute myeloid leukaemia (AML), there are no firm criteria for predicting response to induction treatment of individual patients. *Aims*. To predict the individual response in a clinically relevant time, we analysed the clearance of peripheral blasts (PBC) in 30 AML patients during '3+7' induction course. *Methods*. By extensive flow cytometry (FC), a population of cells with leukaemia-associated aberrant immuno-phenotype (LAIP) was identified in each patient from the initial bone marrow (BM) aspirate. We then obtained LAIP-positive absolute blast counts on peripheral blood (PB) immediately before starting therapy (day 1) and every day until day 8. PBC was expressed as the ratio, converted to logarithmic scale, between baseline value (day 1) and daily absolute blasts count. At day 14, FC analysis was performed on BM in order to identify LAIP-positive residual blasts. The degree of BM clearance was expressed as the ratio, converted to logarithmic scale, between the percentage of LAIP-positive blasts determined at diagnosis and day 14 (LD14). *Results*. Between May 2004 and January 2006, 30 consecutive newly diagnosed non-M3 AML patients aged less than 66 years entered the study and were evaluable for BM response. After a single course, complete remission (CR) was achieved in 17 patients. CR was not obtained in 13 patients (NCR), 8 of whom were refractory. According to conventional criteria (cytogenetics and secondariness) there were 11 high risk patients, of whom 4 achieved CR; 14 intermediate risk patients, of whom 8 achieved CR; 5 low risk patients, all of whom achieved CR. The ranges of distribution of PBC had minimal overlap between CR and NCR groups. Since in patients who achieved CR, by day 7 or 8 blasts were often already undetectable, we excluded these time-points from analysis (Figure 1A).The medians of log reduction in the two groups were significantly different on each day (Figure 1B). The rate of PBC appeared higher in CR than NCR patients with an estimated difference between groups equal to 0.26 (95% CI:0.15-0.37; p value<0.001). This difference was not attributable to differences in baseline PB leukaemic burden and assigned risk. PBC showed an excellent correlation with BM response as assessed by morphologic analysis at haematopoietic recovery and by FC on day 14. Specifically CR was not achieved in any of 11 patients who had a PBC below 2 logs on day 5, whereas CR took place in 17 out of 19 patients who had a PBC greater than 2 logs on day 5. Higher values of PBC on each day were associated with larger LD14 (Figure 1C). This correlation was significant on each day and it increased monotonically over days. *Summary/conclusions*.

These data indicate that PB may be in equilibrium with BM in each AML patient, and that PB clearance gives evidence of BM clearance. Therefore, a major treatment outcome may be predicted very early during the induction therapy of AML patients, thus providing an opportunity to tailor treatment modalities since the outset.



1367

PROLIFERATION OR APOPTOSIS INDUCED BY HALOFUGINONE IN ACUTE PROMYELOCYTIC LEUKEMIA CELLS DEPEND ON THE INTENSITY OF TGFβ INHIBITION

L.L. Figueiredo-Pontes,[1] B. Garcia,[1] G. Lima,[1] R. Proto-Siqueira,[1] M.A. Zago,[1] A. Nagler,[2] R.P. Falco,[1] E.M. Rego[1]

[1]*University of So Paulo, RIBEIRO PRETO, Brazil;* [2]*Chaim Sheba Medical Center, TEL HASHOMER, Israel*

The Transforming Growth Factor-β (TGF-β) is a multifunctional cytokine that plays an important role in cellular homeostasis by regulating cell growth inhibition, differentiation, cellular senescence and apoptosis. A key regulator of TGF-β function is the cytoplasmic isoform of the PML protein. In fact, the inactivation of the PML gene has been demonstrated to cause resistance to TGF-β-dependent growth arrest, induction of cellular senescence and apoptosis. The PML-RARα fusion protein, generated by t(15;17) in acute promyelocytic leukemia (APL), exerts a dominant negative action on PML, and thus indirectly leads to deregulation of TGFβ pathway. However, the cross talk between PML and TGFβ pathways in APL has been poorly characterized. To address this issue, we have analyzed the effect of progressive inhibition of TGF-β activation by Halofuginone (HF), a low molecular weight quinazolinone alkaloid in NB4 cell line. Using a four color flow cytometric method, we simultaneously analyzed BrdU incorporation, cell cycle status, apoptosis and Bcl-2 expression in NB4 cells incubated for 24 hours with increasing doses of HF (6.25, 12.5, 25 and 50 ng/ml). An increase in the percentage of cells in S-Phase was observed with low concentrations of HF (lower than 12.5 ng/ml), whereas higher doses induced apoptosis blocked cell cycle at G2/M and inhibitied of Bcl-2 expression. These results were confirmed by a separated set of experiments in which apoptosis was evaluated by annexin V and propidium iodide labelling and cell proliferation was evaluated by trypan blue exclusion. TGF-β activation was evaluated by determination of its downstream effector *Smad 4 l*evels. These results indicate a bimodal pattern for the effect of TGFβ inhibition in APL. Decrease, but not abrogation, of the activation of this pathway has a proliferative effect, probably by facilitating PML-RARα dominant negative action on cytoplasmic PML. In contrast, complete block of TGFβ signaling leads to apoptosis associated with Bcl-2 inhibition.

1368

CLINICAL SIGNIFICANCE OF MULTIDRUG RESISTANCE 1 (MDR1) GENE EXPRESSION FOR TREATMENT OUTCOME IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

M. Kourti,[1] N. Vavatsi,[1] N. Gombakis,[1] V. Sidi,[2] D. Koliouskas,[2] F. Athanassiadou[1]

[1]*Aristotle University of Thessaloniki, THESSALONIKI, Greece;* [2]*Hippokration Hospital, THESSALONIKI, Greece*

A major cause for early relapse and treatment failure in patients with acute lymphoblastic leukemia (ALL) is the occurrence of multidrug resistance (MDR). One of the mechanisms is the overexpression of MDR1 gene which encodes a drug efflux pump called permeability-glycoprotein (P-gp). The aim of this prospective study was to analyse the expression of MDR1 gene and correlate our findings with clinical, laboratory parameters and treatment outcome in children with ALL. *Material and Methods.* We studied prospectively 49 children with ALL (26 boys and 23 girls) with median age of 5.1 years (range: 18 months to 13.9 years). Four children were also evaluated at initial diagnosis and relapse. All patients were treated according to the BFM95 chemotherapy protocol with a median observation time of 18 months (range 9- 36months). As controls we used bone marrow (BM) mononuclear cells from 7 children who underwent a BM biopsy for diagnostic purposes and was negative for leukemia. Total RNA was isolated from BM samples at initial diagnosis and relapse. The expression of MDR1 and the housekeeping b-actin gene was detected by RT-PCR using the appropriate primers. After electrophoresis of the PCR products in 1.5% agarose gel stained with ethidium bromide, gels were scanned by UV transillumination with a densitometer. The relative mRNA expression of MDR1 gene was calculated using the following formula:

Expression Index (EI): MDR1 PCR product / β-actin PCR product

Results. The mean *MDR1* gene EI was significantly higher compared to the control group ($p<0.05$). The *MDR1* EI in patient samples ranged from 0.02 to 2.49 (median 0.35). Using the median as a cut-off value for high and low expression, high *MDR1* EI was found in 18 (36.7%) patients and their event free survival was significantly worse compared with children with low MDR1 expression (86.67% vs.55.56%; p log-rank: 0.03). High expression of *MDR1* gene did not correlate with immunophenotype, NCI risk classification, white blood count, prednisone response on day 8, and LDH value. Interestingly, significantly higher *MDR1* EI was found at relapse in four paired samples compared with diagnosis. Cox regression analysis revealed that children with high MDR1 expression at diagnosis had a relative risk of 3.36 (range: 1.02-11.46) for failure to achieve a complete remission or relapse ($p=0.04$). *Conclusion*: The expression of the MDR1 gene in childhood ALL is a useful tool in assessing the risk of treatment failure and early relapse and can be used as an additional prognostic factor.

1369

PERIPHERAL BLOOD B-LYMPHOCYTE SUBSETS AFTER TREATMENT OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

B. Mazur,[1] T. Szczepanski,[1] I. Olejnik,[2] D. Sonta-Jakimczyk[1], E. Machura[1]

[1]*Medical University of Silesia, ZABRZE, Poland;* [2]*Childrens' Hospital, CHORZOW, Poland*

The immunosuppressive effect of cytotoxic drugs, basic therapeutic agents in the treatment of childhood acute leukemias, requires monitoring of the immune system following cessation of therapy. The aim of the study was the examination of the relative and absolute numbers of CD19+, CD23+ and CD5+ B lymphocytes in peripheral blood in children with acute lymphoblastic leukemia (ALL) after cessation of chemotherapy. The examined group included 150 children with standard-risk ALL treated with standard chemotherapy protocol. The analyses were performed directly after therapy in 30 children, in 30 children - 3 months later, in 30 children - 6 months later, in 30 children - 9 months later, and in 30 children - 12 months after therapy cessation. The control group consisted of 30 healthy age-matched children. Lymphocyte populations were analyzed by multiparameter flow cytometry with 3-color analyses. Phenotypes of B-cell subsets were obtained with anti-CD19 antibody plus combinations of FITC and PE-labeled antibodies specific for CD5, CD23. The data were acquired and analyzed with Cell Quest software (Becton Dickinson). The proportion of lymphocytes stained with each monoclonal antibody was converted to the absolute per microliter by multiplying the absolute number of lymphocytes per

microliter derived from complete blood count. The study confirmed that the intensive chemotherapy significantly decreases the absolute number of CD19+, CD23+ and CD5+ B-lymphocytes. Nevertheless, the period of 12 months after cessation of the chemotherapy is sufficient to recover the immune system function in all examined standard-risk ALL children.

1370

A TOXIC EFFECT OF METHOTREXATE ON THE BRAIN OF CHILDREN WITH ACUTE LYMPHOBLAST LEUCOSIS

K.V.V. Vilchevskaya

Institute of Urgent and Recovery Surgery, DONETSK, Ukraine

In treating acute lymphoblast leucosis (ALL) in children in terms of ALL-BFM-90(m) program, methotrexate (MTX) utilized intravenously, 1 g/m^2, combined with an intrathecal injection penetrates the hematoencephalic barrier and harms the brain. Purpose. Disclosure of early adverse brain reactions. A bioelectric brain activity (BEAB) was studied using electroencephalogram (EEG) *Nihon Kohden*, Japan. Peculiarities of the brain blood supply were investigated utilizing transcranial ultrasound dopplerography (TUSDG) *Logidor 4*, *Kränzbuhler*, Germany. Quantitative assessment of the EEG values was done through the program of carting the DX-complexes-4000, including spectral analysis on the basis of Fourier transformation. Statistical processing and comparing the EEG file data in the groups was carried out by t-criteria of equality of averages. Study included 32 children, aged 8,1 to 13,5. Following the program polychemotherapy ALL-BFM-90(m) they received protocol 1 and achieved remission. Each child was subjected to EEG 12 times: a day before the administration of MTX; following an intravenous injection of 1/10 dose; three hours later; at the end of 1-8 and 13 days. The linear blood flow rate (LBFR) in the arteries of basis cerebri and the blood flow rate in the direct sinus were studied prior to administration of MTX, a day following the injections and at 5 and 13 days. After injection of 1/10 MTX, negative dynamics of BEAB progressing in 2-3 days was noted in all patients' occipital lobes, i.e. an increase in delta-range slow waves and *acute slow wave* complex. Positive dynamics was noted seven days later. A large percentage of all the rhythms was registered in the right hemisphere before injecting MTX, shifting to the left hemisphere after injection of 1/10 dose. Large representation of rhythms' in the left hemisphere kept up the following 5 days. An average LBFR was normal before injection, one day later it authentically (p<0,05) decreased on both sides, increased 5 days later, and at day 13 there was still a deficiency of blood flow. Blood flow rate in direct sinus increased authentically (p<0,05) a day after injecting MTX, which was a result of venous outflow impairment from the brain surface along the ponticular veins into the upper sagittal sinus, suggesting the venous outflow passed through the deep veins of the brain and the direct sinus. At day 13 the outflow restored, however, did not return to norm. The EEG analysis revealed a buildup of δ-range slow waves and appearance of the *acute slow wave* complexes in the occipital regions after an intratecal injection of MTX, indicating an increase in excitability of the mesodiencephalic structures. The right hemisphere was dominant before and the left after injecting MTX as a result of a set of complementary reciprocal interaction of the hemispheres and the right hemisphere having damper effect on the left one. The fall of LBFR in the arteries of basis cerebri and impairment of the venous outflow was likely the result of dilatation of the brain vessels. Thus, MTX therapy is aggressive to the children's brain and produces substantial changes in the vascular system adversely influencing BEAB.

1371

RELATIVE QUANTIFICATION OF GLUCOCORTICOID RECEPTOR ISOFORMS MRNA IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) A PRELIMINARY REPORT OF 13 CASES

H. Hamidah,[1] A. Maha,[2] N.H. Hamsur,[3] A. Hamidah,[4] Z. Syahril,[3] A. Zarina,[4] O. Ainoon,[4] A.R. Eni,[5] M.I. Hishamshah,[5] R. Jamal[3]

[1]*National University of Malaysia, KUALA LUMPUR., Malaysia;* [2]*Faculty of Medicine and Health Sciences,, KUALA LUMPUR, Malaysia;* [3]*UKM Medical Molecular Biology Institute, KUALA LUMPUR, Malaysia;* [4]*HUKM, KUALA LUMPUR, Malaysia;* [5]*HKL, KUALA LUMPUR, Malaysia*

Backgrounds. Acute lymphoblastic leukemia (ALL) is one of the commonest cancers in children. Combination chemotherapy remains the first line of treatment for ALL and glucocorticoids such as prednisolone and dexamethasone are amongst the key drugs in the regime. The effects of glucocorticoids are mediated by the binding and activation of their intracellular receptor (GR), a member of steroid receptor superfamily. The ability to respond to the treatment is determined by concentration of GR per cell. Alternative splicing of glucocorticoid receptor (GR) gene results in several isoforms namely the GR-α, GR-β, GR-γ and GR-P. The quantity of the GR isoforms mRNA in leukemic blasts has been reported to be correlated to the ALL phenotype and also their sensitivity to glucocorticoid. GR-α is a functionally active receptor and GR-β does not bind glucocorticoid and may have a dominant negative effect on GR-α. Reports have shown that GR-α/GR-β ratio is decreased in resistant patients. However the association of the expression pattern of GR isoforms with their sensitivity to glucocorticoid in childhood ALL in Malaysia is still unknown. *Aims.* The aim of this study was to determine the relative expression pattern of glucocorticoid receptor (GR) isoform mRNA in newly diagnosed and relapsed childhood ALL cases treated in a tertiary hospital in Malaysia. *Methods.* Blasts were isolated from total of 13 cases of childhood ALL, of which 6 were at initial diagnosis, 6 at relapse and one case with paired samples at both diagnosis and relapse. Total RNA was extracted from cell pellets of leukemic blasts. The relative mRNA expression of GR-α and GR-β, was determined by quantitative RT-PCR using fluorogenic Syber Green 1. The quantitative value is reported as 2-DDct, which gives the mean fold change in gene expression normalized to GAPDH as an endogenous control, and relative to the mRNA expression of blast from non-malignant disorders in children. *Results.* The results showed that the relative expressions of GR-α and GR-β in newly diagnosed ALL were 0.94 and 0.32 fold respectively, and for relapse ALL were 0.36 and 0.16 fold respectively. The GR-α/GR-β ratio was higher in the newly diagnosed ALL group (2.93) while the ratio was lower in the relapsed cases (2.25). In the only case of ALL where paired samples were analyzed, the GR -α/GR-β ratio was also reduced at relapse (5.37) compared to at diagnosis (7.55). *Conclusion*: The GR-α and GR-β are expressed in our acute leukemic cases. The GR-α/GR-β ratio in newly diagnosed ALL was lower in our relapsed ALL group compared to the newly diagnosed ALL cases, as previously reported. However, further studies must be performed in a larger group of ALL cases, to correlate the glucocorticoid response with these GR isoforms expressions.

1372

CASE REPORT: HEARING IMPAIRMENT AS THE SOLE SYMPTOM OF ALL-RELAPSE; CAUSED DOCTORS DELAY

S. Einarsdottir,[1] M. EEG-Olofsson,[2] C. Müller,[2] P.O. Andersson,[3] M. Brune[3]

[1]*Sahlgrenska University hospital, GÖTEBORG, Sweden;* [2]*Department of Audiology, SAHLGRENSKA UNIVERSITY HOSPITAL GÖTEBORG, Sweden;* [3]*Department of Hematology, SAHLGRENSKA UNIVERSITY HOSPITAL, GÖTEBORG, Sweden*

Aims. We describe two ALL patients with CNS relapse, demonstrated by gradual hearing impairment, without signs of leukemia in peripheral blood (PB). In one patient, impaired hearing was the only clinical symptom, whereas the second patient presented a combined hearing and vision loss. *Case 1*. A 52-year-old man presented in March 2003 with a Ph+ ALL. Induction therapy included high-dose cytarabine and six prophylactic it injections with methotrexate (MTX). He underwent allo-SCT from an unrelated donor after Cy/TBI conditioning. Only four months post-transplant he relapsed in bone marrow (BM) but attained a second CR after receiving imatinib and donor lymphocyte infusions (DLI). After 10 months of imatinib therapy, and still in molecular CR2 as evaluated by quantitative RT-PCR for the BCR/ABL fusion gene transcript, the patient complained of bilateral, slowly progressing hearing impairment without any other neurological symptoms. He was referred to an audiologist and examination revealed bilateral sensorineural hearing loss with signs of lesions in both inner ear and auditory nerve. A lumbar puncture showed a CNS relapse of ALL. BM aspiration demonstrated both morphological and molecular remission. After it MTX injections, the patient´s hearing improved dramatically. At present, the patient is in molecular remission as assessed both in BM and CSF. *Case 2*. A 16-year-old girl was diagnosed with ALL in February 2000. Induction treatment included six IT injections, maintenance treatment was terminated after two years. In January 2005, with a 4 week history of progressive loss of hearing and finally vision, she was referred to hospital. PB counts and differentials were normal. Blasts with ALL immunophenotype were found in CSF, confirming a CNS relapse. Pure tone audiometry showed bilateral deafness and audiological tests revealed an auditory neuropathy with normal cochlear function..Despite normal PB

counts, a BM aspiration demonstrated an ALL relapse. Systemic and it chemotherapy was administered, however without any improvement of hearing or vision loss. The patient died of septicemia three weeks after admission. *Discussion*. The first case illustrates that bilateral hearing impairment may represent the sole symptom of CNS relapse of ALL. The ominous significance of the patient's hearing problems was overlooked for two months and consequently correct diagnosis was delayed. Interestingly, this patient responded well to it therapy, with improved hearing, and BM disease did not follow CNS relapse. Apparently, imatinib, albeit not protecting the patient against CNS relapse, did prevent fulminant hematological relapse. Also in the second case, there was a substantial doctor´s delay due to patient´s initially seemingly harmless symptoms in combination with normal blood counts. In both cases hearing impairment was caused by neuropathy in the auditory nerve. We conclude that hearing impairment in an ALL patient, even if slow, bilateral and isolated, should strongly raise the suspicion of CNS disease.

1373

HEMATOGONES (B-CELL PRECURSORS) AFTER CHEMOTHERAPY IN PATIENTS WITH ACUTE LEUKEMIA BY 3-COLOR FLOW CYTOMETRY

J. Park,[1] J. Cha,[2] H. Lim,[1] S. Jang,[1] S. Chi[1]

[1]*Asan Medical Center, SEOUL, South-Korea;* [2]*Joongang University Hospital, SEOUL, South-Korea*

Backgrounds. Hematogones, B-cell precursors are present in small numbers in the bone marrow and peripheral blood. The immature mononuclear cells including hematogones in the bone marrow aspirtes should be differentiated from leukemic blasts in the post-chemotherapeutic bone marrow aspirates of patients with acute leukemia. *Aims.* The pattern of hematogones was evaluated to differentiate them from residual leukemic blasts in the post-chemotherapeutic bone marrow aspirates of patients with acute leukemia. *Methods.* The bone marrow aspirates (10 hypocellular marrows, 11 complete remission states, 2 persistence states of acute leukemia, 2 post-BMT states) from 25 cases of acute leukemia (7 AML & 18 ALL) were included to measure hematogones by 3-color flow cytometry (CD19-FITC/CD10-PE/CD34-PerCP). Hematogones were defined as the mononuclear cells with coexpression of CD10 and CD19. We analyzed the patterns of hematogones and the correlation of the proportions of total hematogones & more immature CD34(+) hematogones with patients' ages & the hematologic diagnosis of the bone marrow studies. *Results.* The groups of patients with less than 1% (N=11, group I), 1-5% (N=7, group II), & equal or more than 5% (N=7, group III) of hematogones in the bone marrow aspirates show 14.5 years, 10.1 years & 8.2 years of the mean ages each, and 6.8%, 43.2% & 48.4% of the mean proportions of CD34(+) hematogones each. We could not find any differences of hematogone patterns between AML and ALL, but according to the post-chemotherapeutic bone marrow states the different findings were noted. In hypocellular marrows and in complete remission states, there were 74.0% & 22.1% of the immature or mature lymphocytes, 0.8% & 5.7% of hematogones among nucleated cells and 12.3% & 55.9% of hematogones among B-cells each. *Conclusions.* By 3-color flow cytometry (CD19/CD10/CD34) hematogones could be differentiated from residual leukemic cells in the post-chemotherapeutic bone marrow aspirates of patients with acute leukemia. We found that the hematogones, especially more immature hematogones increase more in the younger patients and that the proportions of hematogones are lower in hypocellular marrows inspite of higher proportions of lymphocytes than in complete remission states.

1374

LATE EFFECTS OF CHILDHOOD ALL TREATMENT ON GONADAL FUNCTION IN MALE SURVIVORS

S. Karaman, I. Yildiz, O. Ercan, T. Celkan, H. Apak, A. Zkan, I. Adaletli, M. Bolayirli, A. Canbolat

Pediatric Hematology-Oncology Department,Cerrahpasa Medical Faculty, Istanbul University, Turkey; Yildi; Istanbul University Cerrahpasa Medical S, ISTANBUL, Turkey

Current treatment protocols in ALL aim survival with minimal effect on fertility. Hovewer, gonadal function is at least finally affected irrespective of treatment age in males with ALL. The aim of our study was to evaluate gonadal function in our male survivors of childhood ALL in the context of pubertal stages. Subjects consisted of 53 males (≥ 9 years old) diagnosed between 1975 and 2002 in our department, after a follow up of 10.07±9.00 years. Twenty-nine healthy males of similar chronogical age (CA) were taken as controls. Mean CA of the study (Group I=G I) and control (Group II=G II) groups were15.65+ 4.79 and 15.42+5.37years respectively (p:0.84). Fourty six patients who had received RT (42; CRT, 2; CSRT) will be indicated as Group IA (GIA). Serum FSH, LH, estradiol (E2), total (T) and free testosterone (fT), inhibin-B, sex hormone binding globulin (SHBG), bilateral testicular ultrasound and semen analysis (in patients > 16 years old, N=24; controls N=9) were evaluated. Pubertal categories (PC) were classified as prepuberty (Tanner stage I=1), early (Tanner stage II-III=2) and late puberty (Tanner stage IV-V=3). Semen analysis was evaluated in the context of categorized sperm counts (azo'oligoazo- normoazospermia) between GI and GII. *Results.* Inhibin-B was significantly lower in GIA than in GII in PC 1. Estradiol and fT were significantly higher and SHBG significantly lower in GI, GIA than in GII in PC 3. In conclusion, despite normal inhibin-B levels in early puberty and late puberty, low inhibin-B levels were found in prepuberty. Our findings suggest it might be due to CRT. High E2 and fT levels and appropriately low SHBG levels in late puberty indicate hormonal levels are not yet compromised in these individuals as also reflected by normal FSH, LH, and T levels. Testicular volumes were not reduced in prepuberty, early puberty and late puberty. Sperm counts were not significantly affected.

1375

LYMPHOMAS IN AIDS

V. Pivnik

Russian Medical Academy of CME, MOSCOW, Russian Federation

For the last 3 yrs we have followed 51 lymphoma -AIDS pts. 96% were drug users and 80% in association with HCV. Non Hodgkin's lymphomas were diagnosed in 37 pts. Male- 23, female- 14, median age 30. CD4 counts were from 20 to 500 (median 300) cells cu/mm. Viral load was from 10000 to 500000 copies /mcL. Histological diagnosis by biopsy and postmortem was received in all pts and immunohistochemistry was performed in half of them. Diagnostic laparotomy for lymph node biopsy was done in 6 cases, thoracotomy in 1, orchectomy in 1, splenectomy in 6 pts. The most often diagnosis to be differentiated from in our cases was TBC, established in 40% of lymphadenopathy-AIDS pts Diffuse B- large cell lymphomas happened in 11, Burkitt lymphoma in 6, MALT- omas in 4, Follicular lymphoma 1, Plasmoblastic lymphoma 1, Castleman's 1, T- cell lymphoma 1, primary CNS Lymphoma in one pts. 16 pts had not received treatment and died soon after admission. 21 pts received CHOP (4 with daunoxome), blocks A-B-C of BFM- NHL- 95 with CNS prophylactics and Mabthera, ESHAP. Complete remissions were reached in 6 pts, died from lymphoma progression 10 pts, 5 pts are on therapy with good response. Hodgkin's lymphoma was established in 14 pts. Male- 11, female- 3, median age 30. CD4 counts from 400 to 1500 cells cu/mm, VL from 1000 to 100000 copies /mcL. Mixed cellular variant was established in most cases 8 pts had not received polychemotherapy because of late admittance and poorest performance status. Chemotherapy. 11 pts achieved complete remission: on COPP, ABVD, BEACOPP2. 3 pts achieved complete remission.1 pt died from TBC in complete remission. 6 pts are on therapy and on HAART with good response. Post treatment CD4 is 1000- 2000 cells cu/mm, VL 1000 to 5000 copies /mcL. Extragonadal germ cell tumors were seen in 3 pts, one female. In one pt 1 yr remission was achieved on BEP therapy. HAART. *Conclusion.* HIV/AIDS pts with malignant lymphomas may receive diagnostical and treatment approaches which in results may be compared with general population. They must have opportunity to enter general hematological service all the country.

1376

RAPID INFUSION OF RITUXIMAB CAN BE GIVEN SAFELY AND HAS A SIGNIFICANT IMPACT ON CAPACITY

C. McCoy, M. El Agnaf, Y.L. Ong

UCHT, BELFAST, United Kingdom

Administration of Rituximab can be associated with infusion related toxicity. The risk is greatest with the first infusion and lower for subsequent infusions. To minimise the risk of reaction stict infusion guidelines have been developed involving lengthy infusion times. With the addition of steroids in combination with Rituximab the risk of reaction may be lower and the length of infusion time shorter. Since April 2005 16 patients in our institution, having RCVP and RCHOP for NHL, received Rituximab at the rapid infusion rate. The first infusion was delivered according to the product monograph. All subsequent cycles were given over a total infusion time of 90 minutes (20% of the total in first 30 minutes, the remaining 80% in 1 hour). The patients were closely monitored

for infusion related reactions. All patients received pre medication of Paracetamol 1 gm orally, Chlorphenamine 10mg IV and Hydrocortisone 100 mg IV, 30 minutes prior to commencing Rituximab. *Results*. All 16 patients recieved the 1st infusion at the standard rate with no adverse effects. To date 75 subsequent infusions have each been administered over 90 minutes. This schedule has been extremely well tolerated with no grade 1- 4 reactions noted. *Conclusion*. The shorter infusion schedule for Rituximab is well tolerated and safe and has had a significant impact on capacity problems in the day therapy unit.

1377

A RETROSPECTIVE ANALYSIS OF 57 CASES OF MANTLE-CELL LYMPHOMA ADMITTED IN THE CLINIC OF HAEMATOLOGY- FUNDENI INSTITUTE BETWEEN 1994 2004

A.C. Colita, I. Ursuleac, M. Vasilica, R. Stoia, E. Niculescu-Mizil, C. Dobrea, A. Dumitrescu, D. Ostroveanu, M. Schmidt, A. Rosca, D. Colita

Fundeni Clinical Institute, BUCHAREST, Romania

Background. Mantle-cell lymphoma (MCL) represents a problem for the haematologist due both to the difficulty in establishing the diagnosis and to the lack of response to standard lymphomas protocoles therapy. *Aims*. The aim of this study was to analyze the clinical aspects, the identification of major prognosis factors as well as the therapeutic results in 57 cases of MCL admitted in the Clinic of Haematology-Fundeni between 1994-2004. *Methods*. The diagnosis was based on the histologic examination (WHO criteria) and/or immunophenotyping tests (CD5+, CD23-). *Results*. Their median age was 61 years, M/F rates 1,2; 81% of patients presented in an advanced stage of disease (stage III-IV Ann Arbor) with generalized adenopathy (70%), splenomegaly (51%) but with a good performance status (75%, with < 2 ECOG). Bulky disease was detected in 26% and extranodal determinations ≥ 2 in 84% of cases. Lymphocytosis ≥ 4.000/μL in 81% and ≤ 10.000/μL in 51% of cases, anaemia (Hb < 12 g/dL) in 50% and bone-marrow involvement in 75% of cases. Other extranodal localizations were recorded in gastrointestinal tract (15%), liver (26%), pleura (17%), Waldeyer ring (7%), skin (7%), orbital space (1%) and Nervous Central System (1%). LDH had increased values over normal limits in 64%. M.component in blood in 14%. 68% had an IPI score > 2 and 32% an IPI score ≥2. The initial therapy started with Chlorambucil+Prednison (16 cases); CVP/COP (21 cases) and CHOP or CHOP-like (21 cases). Fludarabine+Cyclophosphamide were introduced only in relapsing or in refractory diseases in 9 cases. Rituximab was administered in 3 cases (one administration per week) and R-CHOP-like was given in 1 case. Splenectomy was carried out in 3 cases. α interferon was applied in 4 cases (3 administration x 3 MU each per week) as a maintenance therapy. In 23 cases (40%) complete and partial remissions were obtained. The median survival time for the whole lot was 20 months (3 - 103 months). The univariant analysis revealed that good performance status (ECOG L2), limited clinical stages (I, II) no bulky disease, hemoglobin level >12 g/dL, normal values of LDH and IPI < 2 were major predictive factors correlated with long survival. *Conclusions*. Mantle cell lymphoma remains still a problem of diagnosis and therapy; the evolution is ineluctable fatal as the disease is largely disseminated at presentation, is generally resistant to standard therapy with a large percent of relapses which imposes an urgent need for new and more-effective therapeutic procedures.

1378

CLINICAL OUTCOME OF EXTRANODAL MALT LYMPHOMA: A RETROSPECTIVE STUDY OF SINGLE CENTER EXPERIENCE

F. Vural,[1] S. Ocakci,[2] S. Dubova,[2] G. Saydam,[2] S. Cagirgan,[2] Y. Anacak,[2] L. Koenderman,[3] M. Tombuloglu[2]

[1]*Ege University, Medical Faculty, IZMIR, Turkey;* [2]*Ege University, IZMIR, Turkey;* [3]*UMC Utrecht, UTRECHT, Netherlands*

Background and Aims. Mucosa-associated lymphoid tissue (MALT) lymphoma was first described by Isaacson and Wright in 1983 and recognized as a distinct lymphoma with clinicopathologic features in Revised European American Lymphoma (REAL) and World Health Organization (WHO) classification systems of lymphoma. Far from being rare, MALT lymphomas account approximately 8% of all non-Hodgkin's lymphomas, being third most frequent histologic subtype. MALT lymphomas mostly localized to stomach but also were described in various non- gastrointestinal sites such as salivary gland, thyroid, skin, orbit, lung, breast, and kidney. In these sites, MALT lymphoma arise from extranodal sites, often in the setting of chronic inflammatory disorders in response to either infection such as Helicobacter pylori (HP) gastritis in the stomach, or autoimmune process like Hashimato's thyroiditis, Sjorgen's syndrome. We conducted this study to demonstrate our experience in patients with MALT lymphomas and compare our results with the literature. *Patients and Methods.* We retrospectively studied 23 patients with MALT lymphomas of different localizations, treated with different modalities over a period time ranging between 1992 and 2005. The female/male ratio of patients was 15/8 with a median age at diagnosis of 56 years (range 27-88 years). At diagnosis 93% of patients had good performance status (ECOG<2) and 5 (22%) had B-symptoms. 7 patients (30%) had anemia and 12 patients (52%) had elevated levels of LDH. 16 patients (70%) with stage I and II, 7 patients (30%) with stage II and IV were admitted. 12 (52%) of gastric localization of MALT lymphoma and 11 (48%) non-gastric localization (7 salivary gland, 2 lung, 1 thyroid, 1 colon) were observed. None of the patient had bone marrow involvement. *Results.* All the patients are alive with a median 28 months (range 2-150 months) of follow-up. 4 patients (17%) received doxorubicin based systemic chemotherapy, 4 patients (17%) radiotherapy and 2 patients (8%) antibiotherapy for HP eradication only. 10 patients (43%) were treated with complete surgical excision (3 stomach, 5 salivary gland, 1 lung, 1 thyroid), 4 of them combined with chemotherapy (3 stomach, 1 salivary gland), 6 of them combined with radiotherapy (4 salivary glands, 1 thyroid, 1 lung). 2 patients with lymphoma of stomach localization were treated with antibiotherapy followed by localized radiotherapy. One patient has been followed-up alive without treatment because he didn't accept treatment. All the treated patients achieved complete remission (95%) except one who achieved partial remission. *Summary/Conclusions.* Because of the indolent course the prognosis of MALT lymphoma was good regardless of the treatment modalities. The optimal management of MALT lymphomas has not been clearly defined. The treatment choice should be patient-tailored, taking into account the site, stage, age and other clinical characteristic of patient.

1379

EVALUATION OF PERIPHERAL BLOOD AND BONE MARROW INVOLVEMENT IN MANTLE CELL LYMPHOMA (MCL) -IMMUNOPHENOTYPIC AND MORPHOLOGICAL FINDINGS

M.B. Bakrac, N.D. Kraguljac, J.B. Bila, B.M. Mihaljevic, I.E. Elezovic, M.P. Petrovic, M.G. Gotic, A.V. Vidovic, M.P. Perunicic, M.C. Colovic, N.S. Suvajdzic, O.M. Markovic, D.B. Boskovic

Institute of hematology, BELGRADE, Serbia and Montenegro

MCL is a B-cell malignancy with distinct molecular genetics and pathological features. It has been reported that advanced Ann-Arbor stage IV and especially leukemic phase of the disease are associated with poorer prognosis. This study aimed to evaluate immunophenotypic profile of lymphoma cells in bone marrow (BM) and/or peripheral blood (PB) in MCL patients (pt) with clinical stage (CS) IV and V (leukemic phase) in comparing with morphological and clinical features. Forty pts were studied (med. age 62, range 37-82 y., M/F=1.93:1) from 1996-2005. Overall survival (OS) for all pts was 21.8±18.7 [4-82] months. Fourteen pts (35%) were staged in the IV CS and 26 pts (65%) had the leukemic phase of the disease. Presenting features included splenomegaly (75%), hepatomegaly (19%), lympadenopathy (50%), gastro-intestinal and sinuses involvement (6%), respectively. Mean hemoglobin (Hb) was 111±24 g/l, platelets 131±9 $\times 10^9$/l, and leukocytes 51 ± 66 $\times 10^9$/l. The pattern of marrow biopsy involvement was diffuse (47.5% pts), interstitial (30% pts), nodular (15% pts) and paratrabecular (7.5% pts). Morphological blastoid variant of MCL was found in 7 pts (17.5%), whereas the rest of pts had small cells and standard MCL cells morphology. Immunophenotyping and multiparameter flow cytometry were done on the PB (87.5%) or BM (12.5%) samples. Surface markers were identified by using monoclonal antibodies against CD19, CD20, CD22, CD10, FMC7, CD5, CD23, CD38, CD79b, CD2, CD3, kappa, and lambda antigens (Ag). Immunological markers showed a typical expression pattern in all patients: CD19+, CD20+, CD5+, Cyclin D1+, CD23-. Spearman pairwise positive correlations between expression level of CD19 and CD20, CD22, CD5, CD79b Ags, were found (p<0.05). Also, there was a strong positive correlation between expression level of CD38 and CD79b Ags (p<0.05) as well as between CD22 and FMC7 Ags (p<0.05). Presence of blastoid variant of MCL was associated with higher proportion of cells with CD23 Ag expression, comparing to pts who hadn't blastoid variant of the disease (22. 3% vs. 5.9%, Wald - Wolfowitz, z = 3.926, p<0.01). There was a significant predominance of positivity for following Ags: CD22 (73.5% pts), FMC7 (76.19% pts), CD79b (94.4% pts), and CD38 (86.7% pts) among the whole group of pts (Hi square test, p<0.05). The results of the current study demonstrated that among the whole group of pts with CD5+,

CD23- phenotype, pts with blastoid variant had significantly higher proportion of cells with CD23 expression, despite of pts with standard cytomorphology.Differences in immunophenotype between pts with blastoid variant and small cells or typical MCL cells, deserve prospective analyses in large cohort of pts, and give some insights about their biological features.

1380

IS GASTRECTOMY NECESSARY FOR NON-HODGKIN WITH GASTRIC INVASION?

T.K. Kato

Gifu University School of Medicine, GIFU-CITY, Japan

CHOP or R-CHOP has been established as a standard first-line chemotherapy for non-Hodgkin lymphoma (NHL). On the other hand, gastrectomy with postoperative chemotherapy for NHL of stomach has been considered as a standard therapy same as for gastric cancer. Recently, there are some reports that chemotherapy and radiation therapy (RT) for NHL of stomach has same or more survival rate compared with gastrectomy. In this study, we investigated the utility of chemotherapy and the necessity of gastrectomy for the NHL patients with gastric invasion From 1998 January to 2005 October, 91 NHL patients were admitted to our hospital. With endoscopic examination, they were grouped in NHL groups with gastric invasion (GI) (n=13) and without gastric invasion (NGI) (n=78). The average of GI age was 65± 10, and NGI was 62± 15. Gender (M:F) was GI (7:6) and NGI (44:34). PS(0,1:>2) was GI(9:4) and NGI(46:32). According to Ann Arbor classification, Grade I and II were defied as a mild group, Grade III and IV were defied a severe group. Severity (mild: severe) was GI (2:11) and NGI (26:52). Pathological diagnosis of DLBCL was 10 cases (77%) in GI, 54 cases (69%). Average of blood Hb was 11.7±1.1 g/dl in GI and 11.8±1.8 in NGI. Average of albumin was 3.6±0.6 in GI and 3.5±0.6 in NGI. CHOP and R-CHOP were given in 6 cases and 7 cases of GI, 69 cases and 9 cases of NGI. There were no differences between GI and NGI in background of patients. There was significantly no difference cumulative survival rate in between GI and NGI. Also, in GI group, there was no difference cumulative survival rate in between mild group and severe group. It was suggested that chemotherapy of CHOP or R-CHOP is effective for NHL patients, whether there was gastric invasion or not. Gastrectomy might not be necessary for NHL patients except emergency and special cases.

1381

CEOP RITUXIMAB: AN EFFECTIVE AND SAFE REGIMEN FOR ELDERLY PATIENTS = 75 YEARS OLD WITH DIFFUSE LARGE B-CELL LYMPHOMA

D. Mihou,[1] P. Konstantinidou,[2] D. Platogiannis,[2] F.R. Patakiouta,[2] N. Constantinou[2]

[1]*'Theagenion' Cancer Center, THESSALONIKI, Greece;* [2]*'Theagenion' Cancer Center, THESSALONIKI, Greece*

Backgrounds. About one quarter of diffuse large B-cell lymphomas (DLBCL) affects individuals of 75 years of age or older. Nevertheless, data on optimal treatment of these patients is scarce, mainly because very elderly patients are usually excluded from clinical trials due to poor performance status and co-existing medical conditions. According to previous studies, replacement of adriamycin with epirubicin in CHOP regimen has proven equally effective in the treatment of aggressive non-Hodgkin lymphomas (NHL), yielding at the same time lower rates of cardiac and hematological toxicity. Rituximab, on the other hand, is well-known for its effectiveness and good tolerance in the treatment of elderly patients with aggressive NHL. *Aim*. To study retrospectively the efficacy and safety of CEOP regimen ± rituximab in very elderly patients with DLBCL. Methods. Between 1993 and 2005, 45 patients, 20 (44.4%) males and 25 (55.6%) females with median age of 78 (75-85) years were diagnosed with DLBCL in our department. Twenty-one (46.7%) of them had DLBCL of nodal origin and 24 (53.3%) of primary extranodal origin. Twenty-seven (60%) patients presented with early stage (I-II, no X) disease and 33 (73.3%) with IPI 1-2. Nine (20%) patients had B symptoms, 3 (6.7%) bulky disease, 2 (4.4%) bone marrow infiltration and 12 (26.7%) extranodal involvement other than primary. All patients received cyclophosphamide 750 mg/m^2 IV, epirubicin 62.5 mg/m^2 IV and vincristine 1.4 mg/m^2 IV on day 1 and prednisone 75 mg IV on days 1-5. Doses were reduced, mainly due to age > 80 years or medical history of heart disease, by 25% in 15 (33.3%) and by 50% in 3 (6.7%) patients. Rituximab was additionally administered on day 1 of each chemotherapy cycle at a dose of 375 mg/m^2 IV, in 23 (51.1%) patients. Fifteen (33.3%) patients also underwent radiation therapy. All patients were under close hematological and cardiac monitoring throughout treatment. Disease-free survival (DFS), overall survival (OS) and failure-free survival (FFS) were estimated according to the Kaplan-Meier method. Results. Median follow-up time was 30 (1-105) months and median number of chemotherapy cycles administered was 6 (1-8). On an intention-to-treat basis complete response was observed in 33 (73.3%) patients, partial response in 5 (11.1%), stable disease in 2 (4.4%) and progressive disease in 1 (2.2%), while 2 (4.4%) patients were lost before they could be evaluated. Sixteen (35.6%) patients are dead and 27 (60%) are alive, 17 (63%) of which, in remission. Actuarial DFS, OS and FFS rates at 3-years were 75.8%, 67.3% and 53.5% respectively. OS and FFS rates at 3-years were significantly ($p < 0.003$) higher in responders (76.7% and 60.3% respectively) than non-responders (19.8% and 22.9% respectively). No treatment-related deaths were noted, while hematological and cardiac toxicity remained acceptable. Conclusion: CEOP ± rituximab is a feasible, safe and effective treatment for very elderly patients with DLBCL. The high response and survival rates in our study justify the right of these patients to a potentially curative treatment.

1382

EFFICACY OF EPOCH AND RITUXIMAB-EPOCH AS SALVAGE THERAPY FOR RELAPSED OR REFRACTORY B-CELL LYMPHOMA : PRILIMINARY RESULTS OF AN OPENED, NON-RANDOMIZED STUDY IN A SINGLE CENTER

A. Lekhakula, D. Kongkabpan, P. Rujirojindakul

Prince of Songkla University, HAT YAI, SONGKHLA, Thailand

Backgrounds. Relapsed or refractory B-cell Non-Hodgkin lymphoma (NHL) have a poor prognosis. EPOCH chemotherapy and rituximab-EPOCH (R-EPOCH) regimen have been used as salvaged therapies in such cases. *Aims.* To preliminarily evaluate efficacy of EPOCH and R-EPOCH regimens in terms of response rate, survival and toxicities. Possible predictive factors for response were also assessed. *Methods.* Thirty-six patients with relapsed or refractory B-cell NHL were enrolled in Songklanagarind Hospital during January 2003 and January 2006. All of patients received conventional CHOP chemotherapy without rituximab as a first-line treatment. In an opened, non-randomized way, 25 patients received EPOCH (doxorubicin 10 mg/m^2, etoposide 50 mg/m^2, vincristine 0.5 mg as a continuous IV infusion on days1-4, cyclophosphamide 750 mg/m^2 IV on day 5 and prednisone 60 mg/m^2 orally on days 1'5) and 11 patients received R-EPOCH (addition of rituximab 375 mg/m^2 intravenously 24 hr before EPOCH regimen). *Results.* The patient characteristics were not statistically different between EPOCH group and R-EPOCH group, in term of age, gender, histopathology, stage of disease, IPI, B-symptoms, performance status, bulky mass, elevated LDH, prior response to the first-line chemotherapy, and duration from diagnosis to salvage treatment. Of 23 evaluable patients with EPOCH treatment and of 11 patients with R-EPOCH, objective responses were obtained in 52% (35% CR, 17% PR) and 73% (64% CR, 9% PR), respectively (p=.30). There were no significant predictive factors of response as a function of histopathology, stage of disease, IPI, B-symptoms, performance status, bulky mass, elevated LDH, and prior response to the chemotherapy history. Rituximab infusion-related reactions occurred in 2 patients (18%). Febrile neutopenia developed in 12 of 216 cycles (6%). Cardiotoxicity was about 8%. Because of short duration of follow-up (median, 7.8 mo for EPOCH and 10.6 months for R-EPOCH), EFS and OS could not be appropriately analysed at this report. *Conclusion*: EPOCH and R-EPOCH regimens were effective and well tolerated in patients with B-cell NHL who were relapsed from or resistant to the conventional chemotherapy. R-EPOCH seemed to give higher response rate that EPOCH but it is not statistically significant. Because of this preliminary result, more number of patients and longer duration of follow-up are needed.

1383

IN VIVO AND *IN VITRO* PURGING WITH RITUXIMAB PLUS CHEMOTHERAPY, CD34$^+$/CD133$^+$ CELL SELECTION AND HIGH DOSE CHEMOTHERAPY AS CONSOLIDATION TREATMENT IN ADVANCED MANTLE-CELL LYMPHOMA

S. Bassi, F. Gigli, A. Alietti, C. Rabascio, C. Massaro, C. Corsini, L. Saronni, V. Raia, F. Bertolini, L. Calabrese, D. Laszlo, G. Martinelli

European Institute of Oncology, MILAN, Italy

Backgrounds. Mantle-cell lymphoma (MCL) remains an incurable lymphoproliferative disorder with standard chemotherapy (CT). The chimeric monoclonal antibody anti-CD20 Rituximab has demonstrated to improve the CR rate representing an interesting *in vivo* purging modality; however most pts treated with standard CT relapse within 2 years and the role of consolidation therapy is crucial. In vitro purging might contribute in obtaining a molecular (bcl-1) CR which could be predictive

for improved outcome. *Aims.* Here we considered the efficacy of an intensive in vivo and *in vitro* purging consolidation program based on Rituximab with CT, $CD34^+$ positive selection after PBSC collection, followed by HD CT in advanced and refractory MCL pts. Methods. From October 1999 to December 2003 we treated 13 pts (9 newly-diagnosed, 4 previously-treated) with a median age of 55 yrs (range 41-66). At the time of diagnosis 11/13 had IV stage, 5 with extranodal involvement and in 7/13 pts IPI was 2. Molecular bone marrow evaluation showed bcl-1 positivity in 11 pts. Pts received Rituximab (375 mg/m^2) at the first day of each treatment consisting of 2 CHOP-like cycles followed by Cyclophosphamide 4 g/m^2 and G-CSF to collect >2×10^6 $CD34^+$cells/Kg (apheresis were processed by CliniMACS for $CD34^+$/$CD133^+$ cell purification) and then by 2 ESHAP and $CD34^+$ cell reinfusion after Ida 15 mg/m^2 and Melphalan 180 mg/m^2. *Results.* All pts concluded the schedule treatment without any major toxicity and were fully evaluable for clinical response. Before HD CT, 4 pts achieved CR and 9 were in PR. After transplantation 12 pts were in CR and 1 in PR. With a median follow-up of 45 months (range 27-70), 7 pts (54%) are still in CR (confirmed by PCR). Considering the *in vivo/in vitro* purging effect, among the 11 bcl-1 positive pts at baseline, 6 were still bcl-1 positive before stem cell collection; we did not performed *in vitro* purging in 2 pts because of low number of $CD34^+$ collected; 2 out of 4 *contaminated* apheresis became bcl-1 negative after *in vitro* purging. After transplant, 4/7 of pts who received bcl-1 negative apheresis maintain a molecular CR versus 2/4 who received bcl-1 positive apheresis. *Conclusions.* Our data seem suggest that an intensive chemo-immuno consolidation therapy can improve the outcome in advanced MCL pts. Five Rituximab courses as conventional schedule do not appear sufficient to induce a molecular CR; on the other hand the reinfusion of bcl-1 positive cells does not hinder the possibility to achieve a CR and conversely purging *in vitro* can negatively influence the engrafment especially for platelet recovery. The use of different schedules of Rituximab, its employment in the conditioning regimen and as maintenance therapy, could represent a new strategy for pts belonging to this unfavourable risk group.

1384

EFFICACY OF 90Y-IBRITUMOMAB TIUXETAN (ZEVALIN) IN REFRACTORY OR RELAPSED MALT GASTRIC NON HODGKINS LYMPHOMA

P.F. Ferrucci,[1] A. Vanazzi,[1] G. Pruneri,[1] C. Crosta,[1] A. Pinto,[2] C. Grana,[1] M. Chinol,[1] G. Paganelli,[1] G. Martinelli[1]

[1]*European Institute of Oncology, MILAN, Italy;* [2]*Istituto Nazionale Tumori De Pascale, NAPOLI, Italy*

Backgrounds. Radio-immunotherapy (RIT) has been developed to vehiculate radionuclides in a selective manner to tumor cells targeted by monoclonal antibodies. The rationale for the use of this approach consists in the opportunity to deliver radiotherapy (RT) in the tumor bulk avoiding the exposure of other tissues. Toxic effects are well known and limited prevalently to the hematologic counterpart, but transient and reversible. Though, this modality treatment could be offered also to those patients who had previously received RT or who are not suitable to receive it. RIT with Zevalin has demonstrated activity in follicular (FL) and diffuse large B-cell (DLBC) NHL, but data are still lacking in MALT NHL. We report results of a pilot study of Zevalin delivered at standard activity (0,4 mCi/kg) in gastric NHL pts relapsing/resistant after standard therapies. *Aims.* To verify efficacy and confirm safety profiles of standard dose Zevalin in gastric NHL resistant or refractory to conventional systemic treatment. *Results.* From May 2004 to January 2006 7 patients were enrolled. They all had gastric resistant-refractory CD-20 positive B-cell NHL: 2 were DLBC and 5 were MZL MALT. Median age was 57 ys (range 36-64), 3 female and 5 male. At time of treatment 5 out of 7 patients had stage I/IIA (MZL MALT), while 2 out of 7 pts had stage III/IV (DLBCL) of disease. Median number of previous therapies received was 2 (1-4): all of them had received prior CT, 3 prior Rituximab, no one had received prior RT. Toxicities were mild (G2 NCI), reversible after 6 weeks from therapy and primarily hematological. Six out of 7 patients are now evaluable for responses: 4 RC (all MZL MALT), 2 PD (both DLBC NHL). *Conclusions.* Basing on such preliminary results, Zevalin seems to be very active principally in pts with MALT gastric NHL resistant or refractory to conventional systemic treatment. Its mechanism of action mimics conventional RT already known as valid option in MALT gastric NHL. Although the number of patients enrolled is still low, we are continuing our experience in order to confirm such results. However one single administration of Zevalin delivered at standard dosage could be considered a possible alternative option in the treatment of such indolent disease. Updated data will be presented and discussed.

1385

RECOMBINANT URATE OXIDASE (RASPURICASE) FOR THE PREVENTION AND TREATMENT OF HYPERURICEMIA DURING CHEMOTHERAPY FOR HEMATOLOGICAL MALIGNANCIES

P. Konstantinidou, E. Georgiou, E. Verrou, A. Banti, D. Mihou, C. Zervas, N. Constantinou

Theagenion Cancer Hospital, THESSALONIKI, Greece

Tumor lysis syndrome (TLS) is a serious complication of the induction therapy of lymphomas and leukemias. The standard approach for the prevention and management of hyperuricemia is hydration, oral allopurinol and alkalinazation. Urate oxidase is an enzyme that catalyses the conversion of uric acid (UA) to allantoin which is 5-10 times more soluble than UA and therefore is excreted by the kidneys more easily. It is found in most mammals but not in humans. Raspuricase, a recombinant form of urate oxidase catalyses the conversion of uric acid to allantoin and therefore controls hyperouricemia faster and more efficiently than allopurinol. To evaluate retrospectively the safety and efficacy of raspuricase in patients with hematological malignancies who were treated with chemotherapy and had increased risk to develop tumor lysis syndrome. We studied 21 patients (16 men and 5 women) with median age 63 years (range 26-76). They had Burkitt lymphoma (n=3), lymphoblastic lymphoma (n=2), mantle cell lymphoma (n=1), diffuse large B-cell lymphoma (n=5), Hodgkin lymphoma (n=2), chronic lymphocytic leukemia (n=4), acute leukemia (n=4) and received raspouricase during the first therapy for their desease. Before treatment 76% of the patients had increased lactate dehydrogenase (LDH), and 52% increased uric acid. Raspuricase was administered intravenously at a dose of 0.20 mg/kg/day for 2 to 7 days, starting from the first day of chemotherapy. Uric acid, electrolytes, urea and creatinine were measured every day during therapy. All patients responded to therapy with raspuricase. The mean UA level in 11 hyperuricemic patients decreased from 10.7 mg/dl to 0.6 mg/dl after 24 hours. Prophylactic administration of raspuricase to 10 patients with normal UA level reduced the mean UA level from 5 mg/dl to 0.4 mg/dl after 24 hours (for all reductions in UA levels $p<0.001$). We did not observe any increase in the levels of creatinine or any electrolyte disturbances. One patient presented fever as an adverse event which subsided after the interruption of the raspuricase. The results of this study confirm the efficacy and safety of raspuricase for the prevention and treatment of hyperouricemia induced by chemotherapy in patients with hematological malignancies

1386

BODY COMPOSITION CHANGES DURING CHEMOTHERAPY OF NHL

N. Stanisavljevic,[1] D. Marisavljevic,[2] O. Markovic[2]

[1]*University Hospital 'Bezanijska kosa', BELGRADE, Serbia and Montenegro;* [2]*University Hospital 'Bezanijska kosa', BELGRADE, Serbia and Montenegro*

Weight loss, anorexia, and pain are common symptoms in patients with malignancies. Metabolic changes induced in the host lead to loss of adipose tissue and skeletal muscle mass and the known result is poor quality of life and poor response to chemotherapy. The clinical course of a cohort of patients diagnosed non-Hodgkin lymphoma (NHL) and treated with CHOP was evaluated to determine changes in body composition during chemotherapy. The study sample included 30 patients with imunohistochemically confirmed NHL that were treated with CHOP chemotherapy regimen. Before first, third and sixth cycle body weight was measured along with skinfold thickness on four places (by Harpenden caliper) and bioelectric impedance analysis was done (four electrode technique proposed by Hoffer *et al.*) (BIA). Body weight during chemotherapy shows constant but statistically insignificant increase in all patients (mean weight before therapy 69,53kg and after completed therapy 71,61kg). Body fat percentage determined by Durning and Rahaman method shows statistically significant correlation to fat mass percentage measured with BIA that enabled usage of other results computed by BIA. Body fat percentage was (mean measures) 24,85 before therapy and 27,72 before sixth cycle (statistically insignificant increase) in patients with response (ORR 86,7%), and 29,2 to 30,9 in patients with no response to therapy (13,3%). Percentage of lean body mass shows opposite results - decrease during chemotherapy (also statistically insignificant). Patients in this study show unfavorable changes in body composition (increase of fat mass and decrease in lean body mass). Since drugs undergo distribution into various body compartments after administration these changes could be important in well known triad dose-toxicity-response relations.

1387

PLATINUM-BASED PROTOCOLS IN THE TREATMENT OF RELAPSED OR REFRACTORY HODGKIN DISEASE PREVIOUS TO HEMATOPOIETIC PROGENITORS TRANSPLANTATION

J.N. Rodriguez,[1] G. Rodriguez,[2] E. Martin,[2] M.V. Moreno,[2] A. Palma,[2] J.C. Diéguez,[2] M. Carmona,[3] A. Fernandez-Jurado[2]

[1]*Hospital 'Juan Ramn Jimnez', HUELVA, Spain;* [2]*Hospital 'Juan Ramn Jimnez', HUELVA, Spain;* [3]*Hospital 'Virgen del Roco', SEVILLA, Spain*

The platinum-based protocols (ESHAP or EDHAP) have been widely used in relapsed or refractory cases of non-Hodgkin lymphoma (NHL) and even as a conditioning regimen prior to transplantation as well. In contrast to these data, their use in Hodgkin disease is scanty. We present our experience in 5 patients with relapsed or refractory Hodgkin disease treated with these protocols all of them prior to transplantation. Five patients (4 males, 1 female), mean age 41,2 years (25-58) are presented. Histologic subtypes included 3 lymphocyte predominance and 2 nodular sclerosis. Three cases were relapsed and 2 resistant to previous therapies. Four patients were treated with ESHAP cycles and only one with EDHAP. No modifications on the conventional doses or schedule of these protocols were made. In two cases with lymphocyte predominance histology Rituximab (375 mg/m^2, day 1) was added to the conventional protocol. A total of 18 cycles were administered, mean number of cycles was 3,6 (2-5). Four patients received complete doses and in one case corticosteroids were reduced 50% due to previous hypertension. No delayed administration of cycles was observed. Only one patient required G-CSF due to neutropenia. Toxicity included (for 18 cycles): 6 events of grade I-II anemia; 1 case of grade I-II and 3 cases of grade III-IV neutropenia; 4 cases of grade III-IV thrombocytopenia. All neutropenias and thrombocytopenias were observed in the same patient, who presented two febrile episodes as well. Only one patient presented grade I renal toxicity after 3 cycles and other presented hypomagnesemia after 2 cycles. Response was observed in all cases (3 CR and 2 PR). Three patients have received autologous transplantation (other has been proposed but not performed yet), and the other a non-myeloablative allogenic one from his sister. In those cases of autologous procedures enough number of CD34+ cells could be obtained from peripheral blood (2 cases) or bone marrow (1 case) without significative problems. All but one patient (autograft) remain in CR after transplantation. In our experience, platinum-based protocols are a safe, well tolerated and worthwhile option for the treatment of patients with refractory or relapsed Hodgkin disease. These protocols do not seem to affect the number of CD34+ cells collected in cases of autologous transplantation.

1388

HODGKIN'S DISEASE IN CHILDREN: CLINICAL CHARACTERISTICS, TREATMENT RESULTS AND PROGNOSTIC VALUE OF SERUM COPPER LEVEL IN A SINGLE INSTITUTION IN CENTRAL ANATOLIA

T. Patiroglu, M. Karakukcu, M.A. Ozdemir, A. Ozcan, Y. Altuner Torun

Erciyes University, KAYSERI, Turkey

Backgrounds. Hodgkin's Disease (HD) is one of the common malignancies in childhood. There is limited information regarding the management of HD in developing countries. *Aims.* The aim of this study is to analyze clinical characteristics, histology, staging, and treatment results on children presenting with HD and to investigate retrospectively pretreatment factors such as copper level that might influence survival rates. *Patients and Methods.* From March 1988 to May 2004, 42 Turkish children with biopsy-proven HD who were younger than 16 years of age were retrospectively included into the study. The age range of children was 3 to 15 years with a median age of 8.2 years. The male to female ratio (29 male and 13 female) was 2.2:1. Treatment Protocols up to 2000, COPP a modification of the MOPP scheme or ChlVPP regimen were started for 19 patients and radyotherapy were started alone for 5 patients. After 2000, COPP alone for 6 patients and ABVD alternating with COPP for 12 patients were started. *Results.* The main symptom at presentation was painless progressive enlargement of cervical lymphadenopathy (61.9%) and 11 patients (26.2%) had mediastinal involvement. Half of the patients (50.0%) presented with early stages (I and II) and other half of patients presented with advanced stage (III and IV) of the disease. In 17 patients (40.5%) at least one B symptom was present. Mixed cellularity (MC) was the major histopathologic subtype in this series (42.9%), followed by nodular sclerosing (NS)(35.7%), and lymphocytic predominance (LP)(21.4%). A total of 37 children were treated with chemotherapy and additional mediastinal radiotherapy were given to five patients for residual disease. Remission was achieved in 39 (92.8%) of the 42 children who received chemotheraphy or radiotherapy. In the patients who presented with early stage (stage I, II) and advanced stages (stage III, IV) of HD, Overall survival (OS) and 5-year event free survival (EFS) rates were 100.0%, 80.9% and 66.7%, 61.9% respectively. The 5-year EFS was found 100% for patients with low or normal initial serum copper levels and 78% for high initial serum copper levels and the differences was significant (Log rank: 4.61, $p<0.05$). *Conclusions.* In this study, MC subtype was the most common subtype. This feature is different from developed countries. Also high serum copper levels associated with low EFS. This result led us to conclude that the serum copper level may be valuable in prognosis of HD. But this prognostic factor should be validated with large, multicentered prospective clinical studies.

1389

HODGKIN LYMPHOMA IN CHILDREN:THE EXPERIENCE OF 8 YEARS FOLLOW-U (1997-2005)

A. Parxharidou, V. Papadakis, N. Tourkantoni, A. Paisiou, S. Papargyri, A. Kyrantzopoulou, J.P. Panagiotoy, H. Haidas, S. Polychronopoulou

Agia Sofia Hospital, ATHENS, Greece

This is a retrospective study of the paediatric patients cohort with Hodgkin Lymphoma diagnosed and followed 'up in a single center, during the years 1997-2000. The aim of study is to present epidemiological data, clinical characteristics, clinical and histological staging, response to chemotherapy and define the role of irradiation and the final outcome. *Patients and results* : During the last 8 years 24 children, 17 boys and 7 girls, aged 4,5 to 15 years old were diagnosed with Hodgkin Lymphoma in the Department of Pediatric Hematology - Oncology of the 'Aghia Sofia' Children's Hospital. Eight out of twenty-four (8/24) presented with B-symptoms, while 16/24 have been admitted with signs of inflammation of cervical and supraclavicular lymph nodes. The 3 out of 8 patiens with B-symptoms did not presend peripheral lymphadenopathy. In all our patients the diagnosis was established with biopsy material.In all patients bone marrow biopsy was performed. Of note, one of them was diagnosed by bone marrow involvment. Twenty out of twenty - four (20/24) and 19/24 underwent Gallium lymph node and Technetium bone Scann, respectively. Regarding pathology type, 17/24 patients had nodular sclerosing type, six (6) mixed cellularity and one (1) lymphocyte predominant histology. Thirteen (13/24) patients had positive Gallium Scann and while three (3/24) were positive for bone involvement by Technetium Scann. Clinical staging: 3 children were staged as stage I (3/3 IA), 10 stage II (3/10 IIB), 6 stage III (4/6 IIIA) and 5 stage IV. igteneen out of twenty - four (18/24) of the patients were treated according to the German protocol GPOH-HD-95, while 5 were treated with cycles of the MOPP/ABVD regimen . Radiotherapy was administered to the involved sites in 12/24 patients (with bulky disease 3/12, residual masses 3/12, advanced stage 2/12 and local relapse 4/12). Four out of twenty 'four (5/24) experienced relapse (3 stage IV all with mixed cellularity histology and two patients stage IIB with nodular histology). All relapsed patients were treated with chemotherapy followed by autologous bone marrow transplantation. Overall 22/24 children survive today (17 CR1, 2 CR2, 1 CR3 and 2 SD) with a median follow-up 54 months (range 4 to 120 months). One patient is lost follow-up and 1 is dead 24 months after the diagnosis. *Conclusion*. We report the survival of our patients with Hodgkin Lymphomas after their first or second remission is standing high. More patients are salvage following relapse. The longterm follow-up of children with HD concerning possible complications of therapy remains a serious issue. We believe this study offers useful clinical information about staging, appropriate treating decision and outcome of the disease in the longterm inclusion

1390

COMBINATION STUDIES OF LENALIDOMIDE WITH CHEMOTHERAPEUTIC AGENTS ON THE PROLIFERATION OF THE CHROMOSOME 5 DELETED BURKITTS LYMPHOMA NAMALWA CSN.70 CELL LINE

A. Gandhi, J. Kang, P. Schafer, D. Stirling

Celgene Corporation, SUMMIT, USA

Backgrounds. Lenalidomide (Revlimid™), has recently been approved for the treatment of a subset of myelodysplastic syndromes (MDS), and is currently being evaluated as treatment for a broad range of other hematology and oncology conditions, including multiple myeloma, chronic lymphocytic leukemia as well as solid tumor cancers. Lenalidomide efficacy has been reported in clinical trials of MDS patients with a 5q- cytogenetic abnormality, with or without other cytogenetic abnormalities. *Aims.* The present study evaluates the combinatorial effects of

lenalidomide in the lenalidomide-sensitive chromosome 5 deleted Burkitt's Lymphoma tumor cell line, Namalwa CSN.70, with various chemotherapeutic agents used for oncological treatment (cyclophosphamide, doxorubicin, vincristine, methotrexate, cytarabine, ifosfamide, carmustine, prednisone and etoposide). *Methods.* Cells were incubated in 96-well cell culture plates with compounds for 72 hours and assayed by 3H-thymidine incorporation. IC50s were calculated by nonlinear regression analyses with GraphPad Prism. *Results.* Namalwa cell proliferation was inhibited by the chemotherapeutic agents doxorubicin, vincristine, methotrexate, cytarabine, carmustine, prednisone and etoposide. Ifosfamide and cyclophosphamide had no anti-proliferative effects on Namalwa cells. However, lenalidomide in combination with these agents produced varied responses on Namalwa cell proliferation. Specifically, lenalidomide in combination with cytarabine, doxorubicin, or vincristine generated anti-proliferative responses that were equivalent to the inhibition produced by these respective chemotherapeutic agents alone. Lenalidomide in combination with cyclophosphamide or ifosfamide produced anti-proliferative effects similar to lenalidomide alone. These data indicate non-additive effects for the previously mentioned lenalidomide/chemotherapeutic agent combination. However, the lenalidomide/etoposide combination yielded partially additive anti-proliferative effects within the concentration range of 0.05 and 0.5 mM. At higher concentrations, > 0.5 mM, the response became non-additive and comparable to the etoposide treatment alone. Also, the lenalidomide/prednisone combination resulted in partially additive anti-proliferative effects but at concentrations > 0.5 mM and was non-additive at lower concentrations. Antagonistic effects were observed with the lenalidomide/carmustine combination at low concentrations while partially additive anti-proliferative effects were observed at higher concentrations. Antagonistic effects were also observed with the lenalidomide /methotrexate combination. *Conclusions.* Together, these results suggest a possible beneficial anti-proliferative response for lenalidomide in combination with either etoposide or prednisone against diseases such as del 5q MDS.

1391

PROGNOSTIC SIGNIFICANCE OF KI67 AND CLINICAL PARAMETERS IN CLASSICAL HODGKIN'S LYMPHOMA

L.J. Jakovic,[1] B. Mihaljevic,[1] M. Perunicic-Jovanovic[1], V. Cemerikic-Martinovic,[1] A. Bogdanovic,[1] T. Kravic,[2] B. Andjelic,[1] D. Boskovic,[1] V. Bumbasirevic[2]

[1]*Institute of Hematology, BELGRADE, Serbia and Montenegro;* [21]*Institute of Histology and Embriology, BELGRADE, Serbia and Montenegro*

Background. The growth fraction of the malignant cells, International Prognostic Score (IPS), as well as the other clinical and laboratory parameters (bulky disease, tissue eosinophilia and red blood cell sedimentation rate-ESR) are considered to have prognostic relevance in Hodgkin's lymphoma (HL). Aims. To evaluate proliferation index in Hodgkin and Reed-Sternberg (HRS) cells by Ki67 labeling, to determine the prognostic value of other clinical and laboratory parameters include IPS in cohort of patients (pts) with classical HL. Their significance was evaluated regarding response to treatment and survival period. Optimal initial prognostic model was determined according to these findings. Methods. A retrospective study was performed on cohort of 40 pts (20 male/20 female) randomly selected from a large number of treated pts with classical HL, nodular sclerosis subtype. In all pts, initial IPS, presence of bulky disease, tissue eosinophilia and ESR>50 were determined. The median follow-up was five years (yrs). All pts were treated according to standard clinical approach, ABVD regimen. The expression of proliferative marker Ki67 was determined by immunohistochemistry in formalin fixed, paraffin embedded tissue biopsy specimens at diagnosis. We analyzed the percentage of HRS cells with Ki67 positive (+) nuclear staining on 10 different high power microscopy fields (x 400). *Results*. Twenty percent of pts were in stage I and II and 80% were in advanced stage III and IV. The mean age was 35.37±10.3 yrs (75% of pts were <45 yrs). The overall survival rate was 72.5% after 5 yrs of follow-up. Pts with high proliferate fraction (Ki67+ >50%) had worse survival (OS 56%) comparing to those with low proliferation (Ki67+ <50%) with OS 91% (Log Rank test p<0.01). There was not statistically significant correlation between Ki67+ and the achievement of complete remission (p>0.05). Cox's multivariate analysis model revealed that Ki67+ at threshold of 50% was significant independent prognostic factor (p=0.013). From clinical parameters, IPS>3 had a negative trend considering remission rate and overall survival. Bulky disease, tissue eosinophilia and ESR>50 had no significant effect on complete remission rate and overall survival. However, there was trend of divergence in Kaplan-Meier's survival curves after a four years follow-up (log rank p>0.05). *Conclusions*. The patients with IPS>3, bulky disease, tissue eosinophilia and ESR>50 as well as those with high Ki67+ are at risk of relapse and treatment failure, and are eligible for the initial aggressive therapeutic approach.

1392

INCIDENCE AND CLINICAL SIGNIFICANCE OF AUTOIMMUNE COMPLICATIONS IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AND NON-HODGKINS LYMPHOMA

G. Oltean, S. Demian, I. Macarie, M. Candea

Medical Clinic[1], University of Medicine, TARGU-MURES, Romania

Background. Autoimmune complications (AC) as autoimmune hemolytic anemia (AIHA) and immune thrombocytopenic purpura (ITP) can be present in evolution of chronic lymphoproliferative disorders (CLPD) or they, sometimes, precede the diagnosis. The outcome of patients with CLPD associated with AIHA or ITP has been reported, in previous studies, to be similar to the outcome of patients without AC. The aim of our study was to evaluate the incidence and clinical significance of AC in patients with B-cell chronic lymphocytic leukemia (CLL) and in patients with non-Hodgkin's lymphoma (NHL). Methods. In a retrospective analysis we studied 384 patients with CLL (165 pts) or NHL (219 pts) diagnosed and treated in a single institution between 1990-2005. Clinical and hematological data were reviewed for patients with AC, in terms of incidence, therapeutic response, and correlation with disease stage, histological type, and treatment. *Results*. AC were found in 31 of 384 patients (8.07%), in 17 (10.30%) of 165 CLL patients and in 14 (6.39%) of 219 NHL patients. AIHA was present in 18 patients (58.06%), ITP in 7 patients (22.5%), while in 6 patients (19.35%) an Evans syndrome (AIHA associated with ITP) was diagnosed. AC were more frequent in CLL than in NHL (AIHA: 70.58% vs 42.85%; ITP: 29.41% vs 14.28%). In 3 cases (9.67%; 2 CLL and 1 NHL) AIHA was diagnosed before the diagnosis of CLPD. Most of AC appeared in the advanced stages of the diseases (76.47% CLL stage C; 64.28% NHL stages III-IV). The median lymphocyte count at diagnosis, in the 17 patients with CLL, was 48.9×10^9/L (7.8 - 188.1). Of the 14 patients with NHL, AC were diagnosed in 3 cases (21.42%) with small lymphocytic lymphoma, in 2 cases (14.28%) with follicular lymphoma, and in 9 cases (64.28%) with diffuse large B-cell lymphoma. The successful treatment of AC included corticosteroids (96.77%) and/or chemotherapy (19.35%). The outcome of patients with AC was not different from the outcome of patients without AC. Nine (52.94%) of the 17 CLL patients, and 8 (57.14%) of the 14 NHL patients reached a complete response after adequate chemotherapy. The median survival rates were 4.8 years for CLL patients and 3.5 years for NHL patients. No death related to AC was recorded. *Conclusions*. B-cell CLL and NHL are associated with an increased risk of AIHA and ITP, but the incidence of this AC is low. Although most of AC are diagnosed concurrently especially in advanced stages of the CLPD, sometimes they can precede the diagnosis of CLL or NHL. The majority of patients responded well to corticotherapy and/or chemotherapy. The outcome (therapeutic response, survival) and prognosis are similar to other patients with CLL or NHL.

1393

DIFFERENT PROFILES OF ADHESION MOLECULES IN B-CELL NON-HODGKIN'S LYMPHOMA (B-NHL) ARE ASSOCIATED WITH PERIPHERAL BLOOD INVASION

D.M. Matos, E.G. Rizzatti, A.B. Garcia, D.A.P. Gallo, R.P. Falco

University of So Paulo, RIBEIRO PRETO, Brazil

The frequency of the leukemic phase is different in the various histological subtypes of B-NHL. In chronic lymphocytic leukemia (CLL) the involvement of peripheral blood is always present; in mantle-cell lymphoma (MCL), the leukemic phase is present in 30%-70%; and in nodal and splenic marginal B-cell lymphoma (MZL) in about 10% and 50%, respectively. We hypothesized that the down-regulation of some adhesion markers could contribute to the leukemic dissemination observed in some B-NHL subtypes. We evaluated the expression of 10 adhesion molecules in tumor cells of peripheral blood of 17 patients with CLL, 17 with MCL, and 13 with nodal or splenic MZL, all in leukemic phase. All cases of CLL had 4 or 5 points in the Matutes scoring system, while MCL and MZL cases had between 0 and 3 points. In addition, all MCL cases had evidence of CYCLIN D1 overexpression. The mean fluorescence intensity (M.F.I) of the adhesion molecules in tumor cells was measured by flow cytometry in CD19-positive cells. The M.F.I of CD11a, CD11b, CD11c, CD18, CD49c, CD49d, CD29 and CD54 were different in the three groups (Table).

Table: Median values of M.F.I of adhesion molecules.

	CLL	MCL	MZL	p*
CD11a	167.9	257.9	401.6	<0.0001
CD11b	0	21.7	42.7	0.0011
CD11c	46.3	0	143.6	<0.0001
CD18	105.9	150.1	275.2	<0.0001
CD49c	75.5	0	0	0.0002
CD49d	97.9	248	362.1	<0.0001
CD29	117.6	116.6	236	0.0109
CD44	311.4	316.4	285	0.2600
CD54	223.4	238.1	317.2	0.0018
CD62	4.2	10.2	12.1	0.4895

*Kruskall-Wallis test.

The Dunns post test was applied when the p value was <0.05. The comparison between CLL and MCL showed that CLL presented a higher expression of CD11c and CD49c, and a lower expression of CD11b and CD49d. When we compared the CLL with MZL, the CLL showed a higher expression of CD49c and lower expression of CD11a, CD11b, CD18, CD49d, CD29 and CD54. Finally, the comparison between MCL and MZL showed that the MZL had a higher expression of CD11a, CD11c, CD18, CD29 and CD54. The structure of normal lymphoid follicle in lymph nodes depends on appropriate association between the B-lymphocytes and the dendritic follicular cell through the interaction between CD11a and ICAM-1, as well as CD49d and VCAM-1. The lower expression of CD11a and CD49d on CLL cells could facilitate their detachment from the lymph node to invade the peripheral blood. A higher frequency of splenic involvement has been reported in cases of CLL with strong positivity to CD11c. However, in our series 82% of the MCL patients presented with an enlarged spleen, but showed the lowest expression of CD11c among the groups. Thus, our findings give support to the role of adhesion molecules in the determination of nodal or leukemic presentation in lymphoid malignancies.

1394

SAFETY AND EFFICACY OF A COMBINATION REGIMEN CONTAINING PENTOSTATIN, CHLORAMBUCIL AND METHYLPREDNISOLONE IN ELDERLY PATIENTS WITH PROGRESSING CHRONIC LYMPHOCYTIC LEUKEMIA

E. Hatzimichael, A. Vassou, L. Benetatos, L. Bourantas, S.T. Tsiara, E. Kapsali, K. Bourantas

Haematology Clinic, IOANNINA, Greece

Background-Aim: Chronic lymphocytic leukemia (CLL) is a neoplastic lymphoproliferative disorder diagnosed most commonly in the elderly. Alkylating agents used to be the traditional first line treatment, however their inferior response rate and the inability to prolong survival have resulted in purine nucleoside analogues (PNAs) being used as first- and second-line therapy for patients with CLL. Management decisions are more difficult in the elderly because of the increase in toxicity of PNAs in this population. Pentostatin has been proven to be effective and less myelotoxic compared to other PNAs. We wished to evaluate the safety and efficacy of an alternative chemotherapeutic regimen containing pentostatin, chlorambucil and methylprednisolone in CLL patients with progressive disease. *Patients and Methods.* Five elderly, previously multiply treated CLL patients with progressive disease (3 male, 2 female, median age 73.5 years) and one patient with progressing Waldenstrom's macroglobulinemia (WM) were enrolled in the study. Pentostatin was given intravenously at a dose of 4 mg/m^2, days 1 and 15, chlorambucil was given orally at a dose of 10 mg/d, days 1-7 and methylprednisolone orally 32 mg/d, days 1-7. The cycle was repeated every 30 days. All CLL patients had stage C disease (Binet system). *Results.* Four out of five CLL patients responded to treatment. Response was manifested as normalization of the full blood count and significant reduction in lymphadenopathy and/ or organomegaly when previously present. Response was noted at the end of the second cycle. One patient died after the first cycle due to refractory disease. The patient with WM did not respond to treatment and developed grade IV neutropenia leading to discontinuation of this treatment. From the four responders one patient developed grade IV mucositis and delayed further courses and one patient had 3 febrile neutropenic episodes requiring admission. All patients developed at least grade III neutropenia and received GCSF support. *Conclusions.* Combination therapy with pentostatin, akylators and steroids seems to be active in CLL patients with progressive disease. However due to increased toxicity especially in the elderly, we suggest that pentostatin should be given at a lower dose. Addition of appropriate antibacterial, antifungal and antiviral therapy and GCSF support is advisable in order to reduce infection risk in these patients.

1395

RISK OF CANCER IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA. EXPERIENCE AND REPORT FROM A RETROSPECTIVE STUDY IN HEMATO-ONCOLOGY DEPARTMENT, UNIVERSITY HOSPITAL OLOMOUC

J. Vondrakova,[1] R. Urbanova,[1] T. Papajk,[2] Z. Holusova,[2] E. Faber,[2] L. Raida,[2] K. Indrak[2]

[1]*University Hospital Olomouc, OLOMOUC, Czech Republic;* [2]*University Hospital, OLOMOUC, Czech Republic*

Backgrounds. B-cell chronic lymphocytic leukemia (B-CLL) is characterised as a chronic indolent disease with an imunodeficiency. It is the most common leukemia of adult people, especially in the elderly. It is known that imunodeficiency and age can be the risk factors of cancer. Based on these facts we analyzed retrospectively our own data from patients with B-CLL who were diagnosed in our centre from 1994 to 2004. *Patients and Methods.* We analyzed group consisting of 215 patients, male/female 129/86. The median age of patients was 64 (35 - 91), in the clinical stage Binet A 123 (57%) patients, Binet B 55 (26%) patients, Binet C 37 (17%) patients. There were 19 patients who had a malignancy before the diagnosis of B-CLL was determined such as melanoma (4), Grawitz's tumor (3), colorectal cancer (2), basal cell carcinoma (2) and squamous cell carcinoma (1), uterus carcinoma (1), cancer of breast (1), lung (1), stomach (1), prostate (1), parotid gland (1), osteochondroma (1), leiomyosarkoma (1) and urinary bladder papiloma (1). Two patients had two of these tumors as listed above. There were 15 patients who developed second solid tumors after a diagnosis of B-CLL was established and 4 of them did not receive any chemotherapy for B-CLL. These second tumors involved basal cell carcinoma (3), colorectal carcinoma (2), cancer of the thyroid gland (2), lung (2), kidney (2), prostate (1), squamous cell carcinoma (1), uterus carcinoma (1) and bone metastasis (1). The incidence of all solid cancer was in 34 patients (16%), ratio male/female - 20/14 with a median age of 69. Most of these solid tumors were diagnosed in the clinical stage Binet A in 18 patients (53%), Binet B in 8 patients (24%), Binet C in 8 patients (24%). *Conclusion*. The development of second solid cancers in B-CLL diagnosed patients represents a high risk factor and a complication among long term survivors. Longer follow-up is needed to assess proper anamnesis, physical examination (skin lesion included) and diferencial diagnosis.

1396

MULTIPLE MYELOMA IN A PATIENT WITH CHRONIC LYMPHOCYTIC LEUKEMIA. EVIDENCE OF A COMMON PATHOLOGICAL CLONE

A. Lazaridou, S. Mavroudi, E. Verou, D. Markala,

J.I. Christakis

Theagenion Cancer Hospital, THESSALONIKI, Greece

The coexistence of chronic lymphocytic leukemia (CLL) and Multiple Myeloma (MM) in the same patient is very rare. It is uncertain whether the myeloma cell represents a clonal evolution of the CLL cell or a totally different cell population. We present one female, 62 years old patient suffering from CLL. From 1998 to 2005 she has received therapy for the CLL (chlorambucil and fludarabine in combination with cyclophosphmide). On March 2005 she was presented with pancytopenia and the diagnosis of multiple myeloma was established. In the bone marrow aspirate a diffused infiltration from 40% λ- monoclonal myeloma cells and 40% interstitial infiltration from CLL cells was found. The immunophenotype showed the same light chain in both the MM and CLL bone marrow cells examined. The G-banding conventional karyotype and the molecular cytogenetic analysis by M-FISH and M-BAND showed two different clones. One clone with 45 chromosomes and t(13;14)(p11;p11) and an other with the same chromosomal abnormality and additional complex chromosome rearrangements such as deletion of one chromosome 4, t(4;9), t(4;9;15), t(6;9;15), t(8;11), t(8;17), and t(16;21). The patient underwent chemotherapy with thalidomide plus dexamethasone and had a short partial remission of both diseases. Finally, she died on August 2005. Conclusions. The fact that the neoplastic cells carried the same light chain and the presence of translocation

t(13;14) in all 35 metaphases examined while the complex chromosome abnormalities often associated with MM were found in a part of these metaphases suggest that the two diseases were arising from the same neoplastic cell MM being a clonal evolution, probably related to the therapies that the patient had received for the CLL.

1397

IMMUNE THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA: ALEMTUZUMAB (MABCAMPATH) (ANTI-CD52)

B.A.P. Laros-van Gorkom, P.W. Wijermans, P.F. Ypma, M.R. Schipperus

Hagahospital, Location Leyenburg, THE HAGUE, Netherlands

Backgrounds. Chronic lymphocytic leukemia (CLL) is the most prevalent leukemia in adults in The Netherlands. Recent developments with monoclonal antibodies offer new therapeutic options. Alemtuzumab (MabCampath™) is a monoclonal antibody aimed at CD52, which is present on both normal and abnormal B and T lymphocytes. It is indicated as third line therapy in the treatment of CLL, after failure of conventional therapy including fludarabine. The most important side effects of Alemtuzumab treatment are opportunistic infections (pneumocystes carinii pneumonia (PCP) and CMV pneumonitis). It is unclear whether these complications do indeed lead to problems in the treatment of CLL patients in the Netherlands. *Aims.* To gain insight in the use and complications of Alemtuzumab in the Netherlands. Methods. A questionnaire concerning the treatment of CLL patients with Alemtuzumab was made on the basis of the literature [1] and sent to 11 hospitals in The Netherlands Results. From 18-02-02 until 01-04-05 22 patients (mean age 64 years, 16 men, 6 women) with CLL RAI/BINET stadium IIA to IVC were treated with 26 treatments of Alemtuzumab according to schedule (starting dosages 3, 10, 30 mg, followed by 3 times per week 30 mg i.v./s.c. for 4-12 weeks). Patients had received a mean of 3 lines of previous therapy before starting on Alemtuzumab. The time from diagnosis until the start of Alemtuzumab treatment was 5.6 years (4.5) (mean, (SD)). The duration of treatment was 9 (3.4) weeks (mean, (SD)). Reason for early discontinuation of therapy was: fever and other side effects 20%, progressive disease (PD) 13%, complete response (CR) 13%, bone marrow toxicity 13%, other reasons 7%, unknown 33%. 27% of the treatments could be continued for the full 12 weeks. The most prevalent side effects were fever 73%, rigor 42%, dyspnea 19% and tiredness 15%. Infectious complications were pneumonia 26.9% (of which 1 PCP), sepsis 7.7%, herpes zoster 7.7%, sinusitis 7.7%, meningitis 3.8%, guillain barre 3.8%, others 15.3%. The response attained was CR 17%, partial response 35%, stable disease 30% and PD 17%. The duration of the response was 9.5 (7) months (mean (SD)). *Summary/Conclusions.* Treatment with Alemtuzumab is often discontinued prematurely. Therefore the maximal treatment effect cannot be reached. Fear of severe uncontrollable opportunistic infections seems unfounded.

Acknowledgments: C Huisman, dr. S. Wittebol, Meander MC, Amersfoort; dr. J.J. Mol, Rijnstate Hospital, Arnhem; dr. S.J.L. Brada, Jeroen Bosch Hospital, Den Bosch; dr. E. Maartense, Reinier de Graaf Group, Delft; dr. F. de Vries, Slingeland Hospital, Doetinchem; dr. R.M.Y. Barge, LUMC, Leiden; dr. D.H. Biesma and dr. O. de Weerdt, Antonius Hospital, Nieuwegein; dr. W.G. Peters, dr. G.J. Creemers, St. Catharina Hospital, Eindhoven; dr. D.J. de Gooyer, Franciscus Hospital, Roosendaal; dr. H.T.J. Roerdink, 2-Steden Hospital, Tilburg.

References

1. Keating MJ, Coutré S, Rai K et al. Management guidelines for use of alemtuzumab in B-cell chronic lymphocytic leukemia. Clinical Lymphoma 2004;4:220-7.

1398

ANALYSIS OF RISK FACTORS OF 248 PATIENTS WITH B-CHRONIC LYMPHOCYTIC LEUKEMIA AT DIAGNOSIS

E.C. Cmunt,[1] M. Trneny,[1] J. Karban,[1] J. Salkova,[1] K. Michalova[1], Z. Zemanova,[1] L. Pavlistova,[1] J. Schwarz,[2] S. Pekova[2]

[1]*General University Hospital, PRAHA [2], Czech Republic;* [2]*Institute ofHaematology&Blood Transfusi, PRAHA, Czech Republic*

248 patients (139 men and 109 women) with B-cell chronic lymphocytic leukemia were evaluated at diagnosis with respect of clinical stage, CD38 and ZAP-70 expression, cytogenetic changes (by FISH method) and IgVH mutation status and impact of these for overall survival. In Rai 0 and I clinical stage were 203 patients (82,9%), in stage II were 25 patients (10,2%) and in stage III and IV were 17 patients (7,0%). CD38 expression was evaluated at 137 patients (55,2%), positive was at 49 patients (35,8%), negative was at 88 (64,2%) patients. ZAP-70 expression was evaluated at 109 patients (44% from the total number), positive was at 44 patients (40,4%), negative at 65 (59,6%) patients. From 160 evaluations of IgVH mutation status (64,5% of the total number of patients) 71 (44,4%) patients were non-mutated and 89 (55,6%) cases were mutated. From 192 evaluated patients (77,4% of the total number of patients) trisomy of 12 chromosome was present at 22 patients (11,5%), one case was borderline (0,5%), 13(q14) deletion was present at 107 cases (56,6%) out of 189 evaluated (76,2% of the total number of patients), 11(q23) deletion was found at 4 cases (12,9%) out of 31 evaluated (12,5% of the total number of patients), 11(q22.3) deletion was present at 13 cases (16,5%) out of 79 evaluated (31,9%) and 17(p13) deletion by 22 patients (11,4) out of 193 evaluated (77,8%). 48 patients died (19,4%), overall survival in 5 years was 83,5%, in 10 years 58,2%. According to our analysis sex (p=0,0002), Rai clinical stage (p=0,0002), IgVH mutation status (p=0,0001), 17(p13) deletion (p=0,09), CD38 (p=0,05) and ZAP-70 expression (p=0,02) revealed to be significant prognostic risk factors.

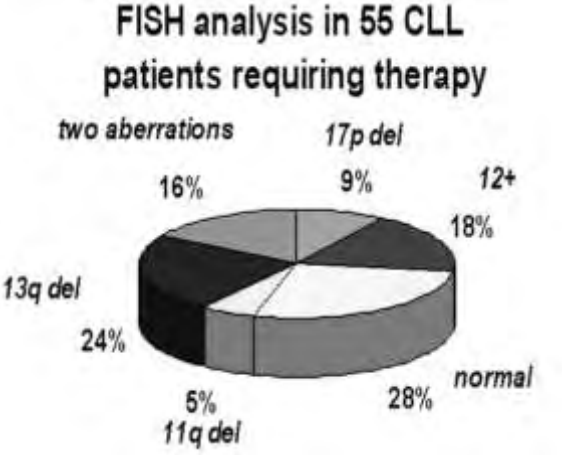
1399

HALF OF CLL PATIENTS REQUIRING THERAPY DISCLOSE NORMAL FISH KARYOTYPE OR THE FAVORABLE 13Q DELETION

L. Shvidel, M. Shtalrid, J. Rosensaft, M. Rosless, M. Haran, A. Duek, E. Sigler, A. Klepfish, A. Berrebi

Kaplan Medical Center, REHOVOT, Israel

Chromosomal aberrations, detected by FISH, are considered as one of the most important prognostic factor in B CLL. Due to its expensive cost we chose to focus our analysis only on patients who required therapy. Fifty-five patients were studied, including 12 patients in stage Binet A and showing progressive disease. The lymphocytes were fixated for analysis before starting cytoreductive therapy. Examination included: 13q, 17p and 11q deletions and chromosome 12 trisomy. The results are presented in figure. The 17p deletions were found in 5 cases, including 2 active PLL patients and 3 CLL patients in stage Binet C. Trisomy 12 was found in 9 patients, all except one exhibited CLL/PL or PLL morphology. The 11q deletions were found in 3 Binet C patients. The 13q deletion was found in 13 patients including 4 in stage Binet A, 5 in stage B, 3 in stage C and 1 with aggressive PLL.



In addition, 13q deletion was associated with 17p del (2 patients), 11q del (4 patients) and 12 trisomy (3 patients). Single case disclosed 12+;11q del. Overall, chromosomal aberrations other than 13 del, were found in 4 out of 12 patients diagnosed in stage Binet A. FISH didn't show any aberrations in 15 cases. Considering the high prognostic significance of FISH analysis in CLL requiring therapy, it would be expected that patients in advanced stages and with progressive disease have unfavorable results. Nevertheless, our analysis in CLL patients requiring therapy showed that FISH results do not always correlate with the clinical stage of the disease. Part of the patients in stage Binet A had chromosomal aberrations supporting the need for therapy, but in other cases FISH revealed a favorable profile. Altogether, half of our patients disclosed either normal FISH results or the favorable 13q del. In conclusion, decision for treatment in patients with CLL cannot rely on FISH analysis alone and should be accompanied by additional prognostic factors.

1400

VH GENE USAGE AND SOMATIC MUTATION DISTRIBUTION CONSISTENT WITH ANTIGEN-DRIVEN SELECTION IN BOTH 'MUTATED' AND 'UNMUTATED' CASES OF B-CELL CHRONIC LYMPHATIC LEUKEMIA

B. Maes, R. Smets, A. Broekmans, S. Franke, G. Bries, V. Madoe, V. Peeters, R. Cartuyvels, J.L. Rummens

Virga Jesse Hospital, HASSELT, Belgium

In B-cell chronic lymphatic leukaemia (CLL), part of the cases shows mutated VH genes indicating that the transformed B-cell has passed through the germinal centre where it has undergone the somatic hypermutation machinery during an antigen (Ag) response. In order to examine the possible role of Ag stimulation in the pathogenesis of B-CLL, we analysed 40 VH sequences derived from 37 CLL patients. VH genes were amplified, sequenced and aligned with all known germline VH genes available on the internet (IgBlast and IMGT). The observed number of replacement (R) mutations within the complementarity-determining regions (CDRs) and the framework regions (FRs) (ObsR CDR and ObsR FR) were compared with the calculated expected numbers (ExpR CDR and ExpR FR), taking into account the inherent replacement susceptibility of CDRs and FRs. The probability (*p* value) that scarcity or excess of R mutations resulted by chance only was calculated for CDRs and FRs using the binomial distribution model. VH gene usage was biased with exclusive use of VH3 (20), VH4 (11) and VH1 (9) genes. VH4-34 and VH3-30 genes were respectively 6 and 4 times used. For 23 of 26 mutated VH genes (homology < 98%), either the *ObsR CDR* was higher than the *ExpR CDR* or the *ObsR FR* was lower than the *ExpR FR* with *p* values < 0,05 in 10 cases, indicating evidence for positive and/or negative selection. Also one *unmutated* VH sequence showed evidence for Ag selection. The preferential usage of certain VH genes as well as the skewed distribution of R mutations indicates that certain Ags may be involved in the pathogenesis of CLL. The VH4-34 gene, most frequently used in this series, encodes for an auto-reactive immunoglobulin (Ig) that is associated with B-cell lymphotropic viruses, particularly EBV. Further studies of the binding sites of restricted Ig, are necessary to elucidate the possibility of Ag involvement in CLL development. Furthermore, as evidence of antigen selection is detected in both *mutated* and *unmutated* CLL cases, its role in predicting prognosis, in addition to the VH-mutation status, should be investigated.

1401

FLUDARABINE-IFOSFAMIDE-RITUXIMAB (FIR): A THERAPEUTIC OPTION FOR CHRONIC LYMPHATIC LEUKEMIA (CLL) PATIENTS RESISTANT TO CHLORAMBUCIL

A. Cortelezzi, M.C. Pasquini, G. Reda, F. Ripamonti, G. Lambertenghi Deliliers

Fondazione Policlinico Universit Milano, Milan, Italy

Ifosfamide is an alkylator belonging to the oxazophosphorins whose efficacy, alone or in combination as mobilizing agent or as salvage therapy, has been shown in solid tumours and lymphomas, but not yet in CLL. Fludarabine is effective in CLL patients refractory/relapsing after chlorambucil and its effectiveness in this disease is enhanced by the association with cyclophosphamide (FC) and monoclonal antibodies, in particular Rituximab (FCR combination). To evaluate the efficacy and safety of a Fludarabine Ifosfamide and Rituximab-containing regimen in a series of CLL patients refractory/resistant to chlorambucil. FIR schedule: Ifosfamide (750 mg/m^2 dd 1-3), Fludarabine (25 mg/m^2 dd 1-3) and Rituximab (375 mg/m^2 d 3) every 28 days. FIR was administered from March 2002 to April 2005 within a monocentric phase II trial to14 patients with advanced CLL (sex: M/F 9/5; age: median 63 yrs; range 51-72 yrs; IWCLL Stage: C in 3 patients, B in 10 patients and A in 1 patient in progression). All patients were refractory/resistant to alkylators and 50% of them have also experienced Fludarabine; 1 patient has also receved Rituximab (previous lines of therapy median: 2, range: 1-2). Patients received a median of 4 FIR cycles (range 1-6). Oral trimethoprim/sulfamethoxazole and acyclovir prophylaxis was given to all the patients during FIR therapy, and two months thereafter. Twelve out of the 14 enrolled patients completed at least 3 FIR cycles. Response was evaluated according to the NCI Working Group criteria on an intention to treat population of 14 patients. An overall response rate (ORR) of 64.2% was achieved, including 7.2% complete response (CR) and 57% partial responses (nodular PR: 28.5%, PR 28.5%). Stable disease was documented in 25% of patients and no progression on therapy was observed. Responses according to site were as follows: peripheral blood 78.6% (CR 64.3%), lymp node 64.3% (CR 35.7%), bone marrow 78.6% (CR: 14.3% nPR 64.3%). Early death was observed in 2 patients (one septic shock during therapy-related neutropenia and one cerebral hemorrage, respectively 10 and 60 days after start of FIR therapy). Grade 3 WHO haematological toxicity was documented in 78% of patients (neutropenia 57%), whereas non-hematologic toxicity was documented in 57% (infection 3 cases, gastrointestinal 3 cases, cutaneous 1 case, renal 1 case). Three out of nine responders have relapsed to date (1 nPR at 14 months and 2 PR at 15 and 26 months, respectively) after a median follow up of 25 months (range 0.5-48 months). Overall survival of non-responders was 11 months (range 0.5-47 months), whereas it was not reached for responders, after a median follow-up of 32 months (range 11-48 monhs). These preliminary data, obtained in a subset of advanced and pretreated CLL patients, showing a sustained and durable response rate with an acceptable incidence of adverse events, suggest that a chemotherapeutic regimen combining Fludarabine, Ifosfamide and Rituximab may be a feasible therapeutic alternative in patients affected by relapsing and refractory CLL.

1402

HAIRY CELL LEUKAEMIA: RETROSPECTIVE CLINICAL STUDY.

A. Cantalapiedra,[1] G. Hermida,[2] M. Dueñas[1], P. de la Fuente,[2] E. Fernandez-Fontecha,[1] O. Gutierrez[1], M.J. Peñarrubia,[1] T. Santoro,[2] J. Garcia-Frade[1]

[1]*Hospital Universitario Ro Hortega, VALLADOLID, Spain;* [2]*Hospital General Yage, BURGOS, Spain*

Background and objectives: The hairy-cell leukemia (HCL) is an infrequent disease with indolent course generally benign and with a good therapeutical response using purine analogues. We have evaluated the presentation form and clinical evolution of this disease. Methods. We reviewed 17 hairy cell leukaemia cases were diagnosed between 1983 to 2005 by flow cytometry and by electron microscope. We revised the clinical evolution and therapeutic response. *Results.* We reported 17 HCL cases, 3 variant form. Mean age of presentation was 60.2 years (28-35). Sex was predominantly male (82.4%) and the more frequent cause of diagnosis was any analytical cytopenia: moderate thrombocytopenia (<120.000 platelets/mm^3) (12 cases), with a mean of 89.000 platelets/mm^3. Leucopenia (<1500 leucocytes/mm^3), was present in 3 cases, granulocytopenia was more frequent (median of 26% with 70% < 1000 granulocytes/mm^3). Anemia (< 12 g/dL of hemoglobin) 8 cases. Splenomegaly at diagnosis appeared in 64.5% of the cases. Asthenia or repeated infections appeared in 58.8% of the patients. The median of HCL cells in blood was 24.9% (1-61%) at diagnosis (15 by flow cytometry and 2 by electron microscope). In relation to treatment and evolution, 2 patients with stable disease did not receive treatment, 10 cases were treated with cladribine, 3 with interferon, 1 with chlorambucil, and 1 with splenectomy. The global response was 94.1% (41% CR, 41% PR and 12% Stable Disease). The median response time was 15 months. The frequency of relapse was high (47.6%) with interferon more frequent with interferon (100%) than with cladribine (33%). However the response to rescue therapy was good (87.5%; 62% with cladribine and 38% with interferon) and persistent. Three patients died but only two in relation with the disease (infectious disease). The median free event survival is 40 months and the global survival 168 months. Conclusions. HCL is an indolent usually diagnosed by analytical methods. The treatment response is high, but with frequent relapses. However the response to rescue is excellent. HCL has a long survival though the age of presentation is advanced.

1403

IMPACT OF TRISOMY 12, DEL(13Q), DEL(17P) AND DEL(11Q) ON THE IMMUNOPHENOTYPE, DNA PLOIDY STATUS AND PROLIFERATIVE RATE OF LEUKEMIC B-CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA

S. Sandra,[1] A. López,[1] S. Barrena,[1] A. Rasillo,[1] J.M. Sayagués[1], M.L. Sanchez,[1] J. Ciudad,[1] P. Giraldo,[2] M. Giralt,[2] M.C. Pérez,[2] M. Romero,[3] L. Perdiguer,[4] A. Orfao[1]

[1]*Centro de Investigacion del Cancer, SALAMANCA, Spain;* [2]*Servicio Hematologia, Hosp. Miguel Servet, ZARAGOZA, Spain;* [3]*Serv.Hematologia. Hospital Ro Hortega, VALLADOLID, Spain;* [4]*Serv.Hematologia. Hosp. Alcaiz, TERUEL, Spain*

B-cell chronic lymphocytic leukemia (B-CLL) is a well-defined clinical entity which displays a variable clinical course in association with the existence of heterogeneous molecular and cytogenetic features. To analyze the relationship between the presence of trisomy 12, 13q-, 17p- and 11q- and the immunophenotype, DNA ploidy status and prolifera-

tive characteristics of neoplastic B-CLL cells. The impact of trisomy 12, del(13q), del(17p) and del(11q) was determined by interphase fluorescence in situ hybridization analysis (iFISH) of purified neoplastic B-cells from a series of 180 patients with newly diagnosed B-CLL on the immunophenotype , DNA ploidy status and the proliferative rate of neoplastic B-cells. Half (50%) of all B-CLL cases studied displayed one (40%) or more (10%) of the genetic abnormalities, trisomy 12 and del(13q) being the most frequently detected ones (23% and 21%, respectively), del(17p) and del(11q) being found in 9% and 9.4% of the cases, respectively. Trisomy 12 was associated with a higher frequency of DNA aneuploidy (p=0.012) together with a higher reactivity for CD22, CD27, CD24 and CD79b. The expression of the this latter marker was also higher among cases with 17p- which in turn showed reduced CD11c expression. Cases carrying del(13q) showed a higher expression of CD5, CD43 and CyBCL2, these latter two markers being also brighter among cases with 11q-. Remarkably, none of the chromosomal abnormalities investigated was associated with an increased proliferation of neoplastic B-cell by itself, although B-CLL cases simultaneous showing 13q- and 17p- displayed a higher percentage of S+G2/M-phase tumor cells as compared with individuals carrying either 13q- (p=0.02) alone or cases showing no genetic abnormalities (p=0.03). In summary, our results confirm and extend previous observations about the frequency of trisomy 12, 13q-, 17p- and 11q- in B-CLL patients, where they affect only a variable proportion of all neoplastic cells showing that the abnormalities have a clear impact on the immunophenotypic profile of B-CLL cells; in contrast, the impact of these cytogenetic abnormalities on the proliferative rate of neoplastic B-cells was only noted for cases simultaneously carrying 13q- and 17p-.

1404

ANGIOGENIC CYTOKINES IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA: ASSOCIATION WITH IGVH MUTATION STATUS AND GENETIC ABNORMALITIES

L. Smolej,[1] C. Andrys,[2] S. Pekova,[6] V. Holubova,[3] J. Sobotka,[3] J. Schwarz,[4] D. Belada,[5] P. Zak,[5] O. Siroky,[5] M. Hrudkova,[5] J. Novosad,[5] J. Krejsek,[5] J. Maly[5]

[1]Charles Univ.Hospital and Medical School, HRADEC KRALOVE, Czech Republic; [2]Institute of Clin. Immunology, HRADEC KRLOV, Czech Republic, [3]J.G.Mendel Oncology Centre, NOVY JICIN, Czech Republic, [4]Institute of Hematology and, BLOOD TRANSFUSION, PRAGUE, Czech Republic; [5]Charles Univ. Hospital, HRADEC KRLOV, Czech Republic; [6]Laboratory of DNA Diagnostics, Hospital Na Homolce, Prague, Czech Republic

Backgrounds. B-cell chronic lymphocytic leukemia (B-CLL) is a disease with an extremely variable clinical course. New prognostic factors such as mutation status of immunoglobulin heavy chain variable region (IgVH) or genetic aberrations detected by fluorescent in situ hybridization (FISH) are being increasingly used in order to identify patients with high-risk disease. Several studies have shown that angiogenesis is increased in B-CLL and may potentially help in prognostic assessment of B-CLL patients. *Aims.* To assess relationship between plasma concentrations of vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF) and IgVH mutational status or genetic aberrations. Methods. We measured VEGF and bFGF using sandwich enzyme-linked immunosorbent assay (ELISA) kits in peripheral blood plasma of 49 patients (males, females, age) with untreated B-CLL and 50 healthy donors. IgVH mutation status was analyzed using cDNA transcribed from B-CLL RNA for touch-down reverse transcriptase polymerase chain reaction (RT-PCR) with degenerate primers for VH1-7 families; IgVH sequences were aligned to the nearest germline using the Ig BLAST database. There were 28 patients with low risk, 17 with intermediate risk and 4 with high-risk disease according to modified Rai staging. Mutated IgVH genes (i.e. more than 2% of somatic mutations) were identified in 23 and unmutated in 26 patients. Genetic abnormalities using fluorescence in situ hybridization (FISH) probes for del 13q, del 11q, del 17p and +12 were investigated in 40 patients. We divided patients according to genetic aberrations into favourable (no abnormality or del 13q, n=25) and unfavourable group (del 11q or +12 or 17p or multiple abnormalities, n=15). Results. There was statistically significant increase of both VEGF (p=0.006) and bFGF (p<0.0001) in patients with B-CLL compared to the control group. Patients with mutated IgVH genes had significantly higher concentrations of bFGF (p=0.0149) but not VEGF (p=0.146) than those with unmutated IgVH. Furthemore, bFGF was significantly higher in both IgVH subgroups (p<0.0001) while VEGF was significantly elevated in IgVH mutated (p=0.0002) but not unmutated patients (p=0.0788). Regarding cytogenetics, significant difference between patients with favourable vs. unfavourable aberrations was neither in VEGF nor bFGF levels (p=0.878 and p=0.494). *Conclusions.* This study confirms that angiogenic activators are elevated in patients with B-CLL. Interestingly, bFGF but not VEGF was significantly higher in patients with mutated IgVH genes in comparison to unmutated IgVH. We did not observe significant difference between low- and high-risk genetic abnormalities. The data must however be interpreted with caution due to relatively low number of patient. Larger study is necessary to perform a more detailed statistical assessment including multivariate analysis.

Funding: Supported by grant NR/8373-3 and research project MZO 00179906 from Ministry of Health of Czech Republic.

1405

CASE REPORT: PLASMA CELL LEUKEMIA SUCESSFULLY TREATED WITH BORTEZOMIB, MELPHALAN, PREDNISONE AND THALIDOMIDE (V-MPT)

N. Pescosta,[1] M. Svaldi,[1] C. Rosanelli,[1] S. Cer,[2] P. Vivaldi,[2] S. Cortelazzo[1]

[1]Regional Hospital Bolzano, BOLZANO, Italy; [2]Ospedale S. Chiara, TRENTO, Italy

Plasma cell leukemia (PCL) is an aggressive disease defined as circulating peripheral blood plasma cells exceeding 2×10^9/L or 20% of peripheral blood plasma cells (1).The disease can occur as primary disease or as secondary disease evoluted of a Multiple Myeloma . The disease is very aggressive with median overall survival of about seven months for primary and 2 months for secondary plasma cell leukemia (2). This is the report of a 67 year old woman with three years history of MGUS (monoclonal gammopathy of unknown significance) IgG lambda that evolved into Multiple Myeloma stage IIA according to Salmon and Durie (International Scoring System ISS 3). Deletion 13q was present in FISH-Analysis. She was treated with induction chemotherapy (3 cycles of Vincristine, Doxorubicine and Dexamethasone (VAD)), stem cell mobilisation with IEV (Ifosfamide, Epirubicine, Etoposide) and double autologous transplantation after conditioning with Melphalan 140mg/m^2. After the second autologous transplantation the patient remained in partial remission showing a small monoclonal component in the serum for seven months when the patient presented at the day hospital with diffuse effusions. Peripheral blood showed 19×10^9/L white blood cells, 8.9 g/dL of hemoglobin and 16×10^9/L platelets with a differential leukocyte count of 50% neutrophils, 22% lymphocytes, 4% monocytes and 24% plasmacells. Flow cytometric analysis confirmed the presence of 36% plasmacells as shown by the expression of CD138$^+$. All of them had an aberrant antigen expression (CD138$^+$/CD19$^-$/CD56$^-$). Further the patient had acute renal failure with a creatinine-level of 4.1g/dl. The monoclonal component had risen to 5g /l. Bone marrow aspiration was not possible (punctio sicca) and histological examination showed an almost complete infiltration of the bone marrow by clonal plasmacells of intermediate differentiation. The patient was initially treated with dexamethasone 40 mg/die on days 1-4, 9-12 and 17-20 for two cycles with reduction of plasmacellinfiltration in the bone marrow to 50% of all nucleated cells. After clearance of plasmacells in the peripheral blood smear cerebrospinal fluid was analysed and the presence of plasmacells was excluded by morphological examination and by flow cytometry. After the two cycles of Dexamethasone-monotherapy a combination chemotherapy including bortezomib (1.3 m^2 on days 1, 4, 8,1 1), thalidomide (50 mg/die), melphalan (0.4mg/kg on days 1-5) and prednisone (40 mg/m^2 on days 1-5) (3) was started. After three of four cycles (recycling every 35 days) the patient had a normal peripheral blood count and the monoclonal component in the peripheral blood disappeared while immunofixation remained positive (near complete Remission nCR) A maintenance therapy with daily thalidomide (50mg/die) and dexamethasone (40 mg/die days 1-4 to recycle every 28 days) was initiated and eleven months after diagnosis of plasmacell leukaemia the patient is still in nCR without significant side effects. Combination therapy including bortezomib, thalidomide, melphalan and prednisone should be considered as effective and save treatment of plasmacell leukemia.

1406

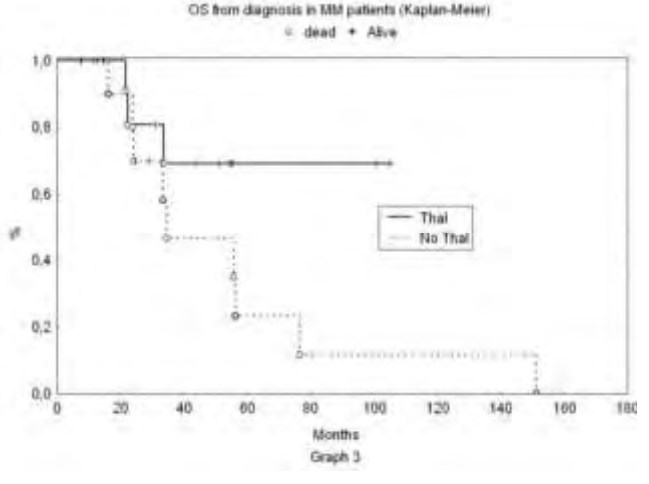
MAINTENANCE WITH VERY LOW DOSE THALIDOMIDE AFTER AUTO-SCT IN MULTIPLE MYELOMA: LOW TOXICITY AND IMPROVED OUTCOME

V. Mettivier,[1] L. Pezzullo,[2] S. Rocco,[2] O. Finizio,[2] G. Nunziata,[2] L. Bene,[2] C. De Rosa[2]

[1]A.O.R.N. 'A,Cardarelli', NAPLES, Italy; [2]Haematology, NAPLES, Italy

High dose therapy with single or double transplantation (auto-SCT) has improved prognosis of multiple myeloma (MM). New drugs are

promising in upfront therapy while the role of maintenance is still debated. Thalidomide (thal) is an active drug in the treatment of myeloma, and is been investigated as first line therapy. It could be useful in the control of minimal residual disease. We used thal as maintenance after autologous transplantation (single or double) and compare the outcome with other maintenance or none. From January 2001 to December 2005 25 patients (13 males and 12 females) with MM have been treated in our institution. Median age was 58 years (range 40-72). 12 were IgG, 7 IgA, 4 light chains and 2 plasma-cell leukaemia. Treatment was 4 cycles of VAD regimen followed by auto-SCT. 9/25 performed double auto-SCT. 3 months after SCT, 13 patients (9 single and 4 double SCT) began thal 50 mg/die as maintenance therapy. 12 patients (7 single and 5 double SCT) received IFN-γ (4/13), dexa (3/13) or no therapy (5/13). The 2 groups were regarding the type of myeloma: 6 IgG, 3 IgA, 3 light chains and 1 plasma-cell leukaemia in the thal group; 5 IgG, 4 IgA, 1 IgD and 1 plasmacell leukaemia in the other. Response to SCT: 3 CR, 7 PR and 1 NR in the thal group; 5 CR, 4 PR and 1 NR in the other. In the thal group 3/11 patient relapsed. Median follow up from the beginning of maintenance therapy was 30 months (range 9-46) with 7/10 patients in CR or stable disease, with a progression free survival (PFS) and overall survival (OS) projected at 47 months of 73%. In the other group, 8/10 patients relapsed. Median follow up was 30 months (range 4-54) with a median PFS and OS of 8 and 18 months respectively. In the two groups the patients in progression have effected, as lifesaving therapy, thalidomide 200 mg / die + dexametasone 20 mg days gg 1-4 and 15-18. The difference between the 2 groups is statistically significant for PFS (p: 0,007), and not significant for OS (p: 0,057) even if difference (73% vs. 10% at 100 months) appears clear (Graph 3). From diagnosis the median OS is of 72 months in no thal group and is 73% projected at 100 months (*p*: 0.2 graph 3) in the thal group. Thal was administered for a median period of 12 months, being neurological toxicity the main reason of suspension (3/10 patients). Neurological toxicity grade I-III was present in 65% of patients, while haematological toxicity grade I occur in 55% of patients. In conclusion, in a small number of patients low dose thal as maintenance after auto-SCT resulted in an improved PFS and OS when compared with other or none maintenance, with acceptable toxicity. Further studies in larger cohorts and randomized trials are needed to confirm this experience.



Graph 3

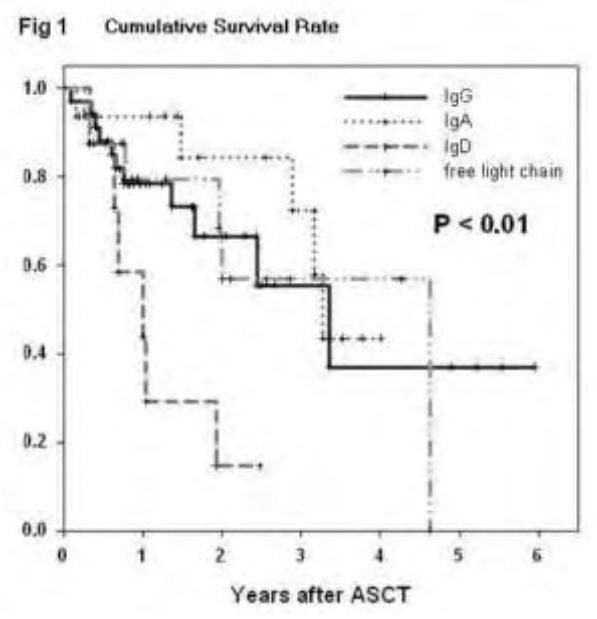
1407

POOR SURVIVAL OF KOREAN IGD MULTIPLE MYELOMA PATIENTS FOLLOWING HIGH-DOSE MELPHALAN AND AUTOLOGOSU STEM CELL TRANSPLANTATION

C. Suh, Y.P. Jeong, S. Kim, Y.H. Cho, J.E. Koo, D. Lee

Asan Medical Center, SEOUL, South-Korea

Backgrounds. IgD multiple myeloma (MM) accounts for 2% of MM subtypes in western countries and has been reported to have poorer prognosis than other subtypes. Aims. The purpose of this study was to compare the survival following high-dose melphalan and autologous stem cell transplantation (ASCT) between IgD MM and other subtypes. *Methods.* Between November 1996 and January 2005, a total of 77 patients with MM who were treated by ASCT at Asan Medical Center were available for analysis. *Results.* A total of 9 patients with IgD MM were identified, accounting for 11% of all MM patients. There were 36 patients (47%) with IgG MM, 17 patients (22%) with IgA and 16 patients (20%) with free light chain. Each MM subtype had similar distribution of SWOG stage at ASCT. With median follow up of 17 months, median overall survival (OS) was 39 months. IgD MM had the lowest survival among the MM subtypes ($p<0.01$). Median OS of IgD MM, IgG MM, IgA MM, and free light chain MM were 12 months, 62 months, 40 months, and 55 months, respectively. Kaplan-Meier survival curve according to MM subtype was as Figure 1.*Summary/conclusion*: In this small-sized single center study from Korea, IgD MM had a poorer survival than other subtype after ASCT.



1408

CHARACTERISTICS OF VASCULAR ENDOTHELIAL GROWTH FACTORS (VEGFS) AND THEIR RECEPTORS GENE EXPRESSION IN PATIENTS WITH MULTIPLE MYELOMA (MM) WITH DIFFERENT RESPONSE TO THERAPY

I. Buravtsova,[1] Y. Sablina,[2] A. Karamysheva,[2] A. Golenkov[1]

[1]*Moscow Regional Research Clinical Istitu, MOSCOW, Russian Federation;* [2]*N.N. Blokhin Rusian Cancer Research Cen, MOSCOW, Russian Federation*

Backgrounds. The correlation between the activity of angiogenesis (microvessel density and VEGF-A expression) and certain clinical characteristics (such as progression and poor prognosis) for patients with MM is shown. VEGF-A is known as the main tumor angiogenic factor, but several other factors of VEGF family (such as VEGF-C and VEGF-D) could also play a role in stimulation of angiogenesis. As compared to VEGF-A, the expression of VEGF-C and VEGF-D, as well as their possible role in MM progression is much less studied. The study of VEGF-A, as well as other growth factors of VEGF family, and their receptors gene expression in patients with MM can bring to discovery of the new proangiogenic target. *Aims.* To investigate the VEGFs (VEGF-A, VEGF-C and VEGF-D) and their receptors (VEGFR1, VEGFR2 and VEGFR3) genes expression in bone marrow aspirates of MM patients. To compare the intensity of gene expression with such clinical characteristics as disease progression and resistance to therapy. *Methods.* Mononuclear cell (MNC) fraction was purified from the bone marrow aspirate of patients with newly diagnosed MM by Ficoll-Hypack centrifugation. Gene expression was evaluated by semi-quantitative RT-PCR technique. *Results.* The gene expression was studied in bone marrow aspirates of 13 patients with newly diagnosed MM, III stage, aged between 50 and 73 years. These patients had the following immunochemical types of MM: 7 patients - Ig G, 1 patient - Ig A, 1 patient - Ig M, 2 patients with Myeloma Bens-Johns, 2 patients had undefined type of MM. All patients had undergone cytostatic therapy according to M-2 protocol. VEGF-A was expressed in 12 patients, 6 of them displayed the high level of expression. One patient showed no expression. VEGF-A expression was absent in MNC of 1 patient, while 12 patients expressed VEGF-C mRNA and the high level of expression was observed in 5 patients. VEGF-D expression was registered in 8 patients (1 of them had high level of expression) and 5 patients were VEGF-D-negative. The intensive VEGFR1 expression was noticed in all 13 patients. VEGFR2 was expressed in one patient only. *VEGFR3* was expressed in 10/13 patients with high levels of expression in 7 patients. The gene expression was compared with the severity of

the clinical symptom manifestation. The only patient with intensive VEGFR2 expression died within 8 months, this patient had the high levels of VEGF-A, VEGF2, VEGFR1 and VEGFR3 expression, as well. Two other patients, who died during 8-12 months of disease progression, also had the high levels of VEGFs and VEGFRs expression. The comparison of VEGFs and VEGFRs expression in patients before and after therapy revealed that VEGF-C expression was stimulated in patients resistant to therapy. *Conclusions.* Our data showed the high frequency and the similar pattern of VEGF-A and VEGF-C expression in bone marrow aspirates of MM patients studied. VEGF-D and VEGFR3 were expressed to the lesser extent. The high level of VEGFR1 expression was registered in all patients, while all patients except one were VEGFR2-negative. Our data suggest that VEGF-C expression could be the predictor of poor prognosis.

1409

UNUSUAL CNS AND CUTANEOUS INVOLVEMENTS IN MULTIPLE MYELOMA DURING BORTEZOMIB TREATMENT

M.T. Pirrotta,[1] A. Gozzetti,[2] A. Bucalossi,[2] A. Cerase,[3] M. Bocchia,[2] F. Lauria[2]

[1]*University of Siena, SIENA, Italy;* [2]*Unit of Hematology, SIENA, Italy;* [3]*Unit of Neuroradiology, SIENA, Italy*

Background. Since last years the proteasome has emerged as a real and exciting target for anticancer therapy. Velcade (bortezomib, PS341) remains the first selective proteasome inhibitor that has demonstrated significant preclinical activity in several tumor models and significant efficacy in patients with refractory or relapsed multiple myeloma. The major biological effect of bortezomib is the inhibition of the nuclear transcription factor NFkappaB, with subsequent inhibition of the tumor cells growth, induction of apoptosis, inhibition of angiogenesis and of cellular adhesion. *In vitro* bortezomib induces apoptosis of multiple myeloma cells and inhibits cell adhesion within the bone marrow microenvironment. The preliminary results of several phase I and II studies showed high antimyeloma activity of bortezomib alone or in combination with cytotoxic agents such as doxorubicin, melphalan, dexamethasone or thalidomide in patients with newly diagnosed multiple myeloma.

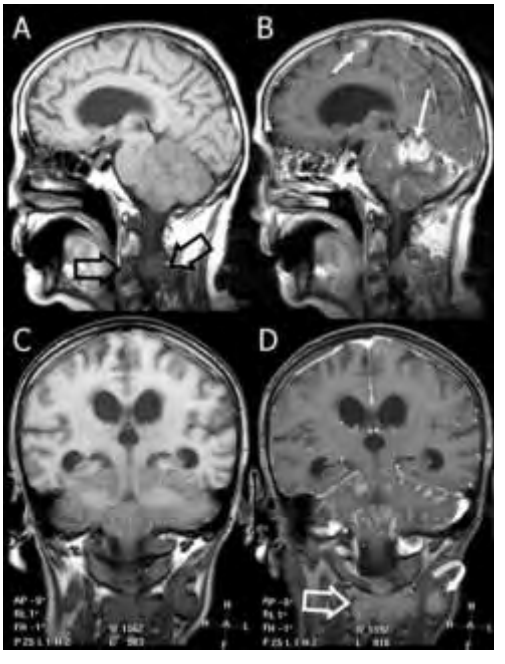


Figure 1. Patient 2. Cranial leptomeningeal myelomatosis.

Methods. We describe two cases of multiple myeloma patients that developed unusual cutaneous and CNS localizations during treatment with bortezomib alone. A 75 years old male patient with immunoglobulin G-kappa multiple myeloma resistant to previous therapies with melphalan and thalidomide recived bortezomib (given day 1, 4, 8, 11 at 1.3 mg/m^2 every 21 days). After two courses a sierologic response (from IgG 7170 mg/dl to IgG 1360 mg/dl) was obtained. However, several cutaneous lesions localized at the face, arms and chest were presented. The hystologic evaluation revealed plasma cells localization, therefore dexametasone was added to bortezomib and a complete disappearance was observed two weeks later. A 74 years old female patient with immunoglobulin G-lambda multiple myeloma resistant to previous treatments with melphalan and thalidomide started a treatment with bortezomib (given day 1, 4, 8, 11 at 1.3 mg/m^2 every 21 days) because of disease progression (IgG 3060). After the first course of bortezomib, while the monoclonal component drastically reduced (IgG 1470 mg/dl), multiple sub-cutaneous nodular lesions and meningeal involvement of multiple myeloma with massive infiltration of cerebellum appeared and the patient died after one week. *Conclusions*. To our knowledge, these are the first cases of cutaneous and CNS localizations of multiple myeloma during treatment with bortezomib. Pharmacokinetic studies have demonstrated that after administration of a single dose bortezomib is rapidly distributed into nearly all tissues, with the exception of adipose tissue and certain tissues in the brain protected by the blood-brain barrier. Our cases show that bortezomib is fastly effective in reducing the size of the disease, but that it can't pass the emato-encefalic barrier and can't reach adipose tissue. Interestingly both patients were previously treated with thalidomide, a molecule that has been recently associated with extramedullary relapses probably because it increases the expression of cytoadhesion molecules such as CD138 and CD56 in myeloma cells. It could be of interest to evaluate in further studies the expression of cytoadhesion molecules also during treatment with bortezomib.

1410

COMBINED ADMINISTRATION OF BORTEZOMIB AND DEXAMETHASONE IN THE TREATMENT OF REFRACTORY MULTIPLE MYELOMA (MM)

M. Sagristani, S. Improta, M. Esposito, A. Lucania, M.R. Villa, L. Petriccione, L. Mastrullo

PO San Gennaro, NAPLES, Italy

Multiple myeloma (MM) is a neoplastic disease especially affecting elder patients even if in recent years it has been also observed in younger patients. The use of the proteasome inhibitor bortezomib has been recently introduced in the treatment of relapsed and/or refractory MM. In fact, Bortezomib has proven to be safe and effective in MM patients not only as monotherapy but also given in combination with cytotoxic agents. Bortezomib-based combination regimens have induced clinical benefits with manageable toxicities and may ultimately lead to improvement in the duration of response and survival of patients in the first-line setting. In our institution we are following 45 patients with stage II/III MM and 7 out of 35 (4 F and 3 M, median age: 71 years, r.: 68-77 years) suspended chemotherapy after 6 cycles of Melphalan and Prednisone regimen for excessive toxicity even if they presented progression disease (PD) at the clinical re-staging performed with both serum marker evaluation and cytological examination of bone marrow blood. All the 7 patients refused thalidomide treatment and underwent a treatment with bortezomib (1,3 mg/m^2 i.v. d. 1,4, 8, 11 every 21 days) together with dexamethasone (40 mg i.v. days 1-4 every 21 days). At a clinical re-staging performed after four courses from the beginning of bortezomib-dexamethasone combined administration a partial remission (reduction of M-component > 50-75%) was recorded in 6 out of 7 patients while the remaining was in steady disease (SD). Thereafter all patients received further four courses of therapy. At one month from the end of treatment two of seven patients achieved a complete remission (negative immunofixation) and the remaining showed a partial remission (PR). At the present, (month +9) only one patient shows a progression disease, while two patients are in CR and four in PR. Our results suggest that the combination of bortezomib and dexamethasone is effective and well tolerated in the treatment of refractory MM in elderly patients. Although there are several published data on the activity of the therapy based on the combination between bortezomib and dexamethasone, little is still known about the improvement in the duration of response and survival of elderly patients in the first or second line therapies.

1411

CLINICAL EFFICIENCY AND TOXICITY OF REGIMENS VAD AND HYPERCVAD IN MULTIPLE MYELOMA

S.I. Moiseev,[1] N.V. Stepanova[2]

[1]*State Pavlov medical university, ST-PETERSBURG, Russian Federation;* [2]*State Pavlov Medical University, ST-PETERSBURG, Russian Federation*

The primary objective of this investigation was to compare the overall response rates and toxicity of the standard regimen VAD-D-D and

regimen HyperCVAD for first line treatment patients with multiple myeloma. First group consist of 40 patients (59±4 years) which received 4-6 standard regimens VAD-D-D every 28 days. Second group consist of 20 patients (57±3 years) which received 4-6 standard regimens HyperCVAD (Cyclophosphamide 300 mg/m^2 i.v. twice 1-3 days, vincristine 2 mg i.v. day 1 and 9, doxorubicine 50 mg/m^2 i.v. day 4, dexametazone 40 mg per os 1-4 and 9-12 days) every 28 days. All patients had poor performance status, elevated LDH values, but did not have low platelet count. An objective response (complete or partial) was documented in 45% and 65% of patients treated with VAD and HyperCVAD, respectively. Hematological and non-hematological toxicities were mild or moderate and equally distributed between the two treatment arms with the exception of neutropenia III-IV, which was more common after HyperCVAD (90%). The duration of neutropenia was from 5 to 9 days. 3 (15%) patients had febrile neuthropenia. Early death was in first and second groups 5% and 0% respectively. Project 3 years overall survival was 65% and 90% in VAD and HyperCVAD groups respectively. These preliminary date suggest that regimen HyperCVAD more effective than standard regimen VAD.

1412

BORTEZOMIB AS SALVAGE TREATMENT IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

D. Mihou,[1] A. Banti,[2] V. Perifanis,[3] I. Katodritou,[2] C.H. Kartsios,[2] E. Verrou,[2] D. Krikelis,[2] C.H. Chatziaggelidou,[2] V. Garipidou,[3] K. Zervas[2]

[1]*'Theagenion' Cancer Center, THESSALONIKI, Greece;* [2]*'Theagenion' Cancer Center, THESSALONIKI, Greece;* [3]*'Hippokration' General Hospital, THESSALONIKI, Greece*

Backgrounds. Bortezomib is a proteasome inhibitor with significant antitumor activity, exerted through disruption of critical signaling cascades that promote cell adhesion and cycle progression, thus inducing apoptosis and decreasing cell proliferation. Bortezomib alone or in combination with dexamethasone has already been reported to yield high response rates and durable remissions in patients with relapsed/refractory multiple myeloma (MM). *Aim:* To evaluate the efficacy and safety of bortezomib as salvage treatment in our patients with relapsed/refractory MM. *Methods.* Between August 2004 and December 2005, 22 patients with progressive MM, 13 (59.1%) males and 9 (40.9%) females, of median age 63 (49-82) years, were treated with bortezomib. Thirteen (59.1%) patients were in relapse and 9 (40.9%) in refractory relapse. Median time from diagnosis was 22 (3-180) months. Patients had previously received a median number of 2 (1-4) different regimens and 5 (22.7%) had undergone high dose therapy with autologous stem cell transplantation. Bortezomib was administered at a dose of 1.3 mg/m^2 IV on days 1, 4, 8 and 11 every 3 weeks. In case of stable or progressive disease after 2 therapy cycles, dexamethasone 20 mg PO was added to the regimen on days 1-2, 4-5, 8-9 and 11-12 of each cycle. Response and toxicity were evaluated according to EBMT and NCI criteria respectively. Remission duration (RD), overall survival (OS) and event-free survival (EFS) were estimated according to the Kaplan-Meier method. *Results.* Median follow-up time was 7 (2-15) months. Patients received a median number of 6 (1-8) therapy cycles. Median time-to-response was 2 (1-5) months. Complete response was observed in 2 (9.1%) patients, very good partial response in 4 (18.2%) and partial response in 11 (50%), yielding an overall response rate of 72.3%. Eight (36.4%) patients are dead and 14 (63.6%) are alive, 12 (85.7%) of which, in remission. Median RD and OS are not reached. Actuarial RD rate at 6 months and OS rate at 12 months was 57.6% and 52.5% respectively. Median EFS was 6 (95%CI: 1-15) months. Peripheral neuropathy was observed in 13 (59.1%) patients, thrombocytopenia in 11 (50%), fever in 9 (40.9%), microbial respiratory infections in 8 (36.4%), herpetic skin infections in 4 (18.2%), skin rash in 4 (18.2%), diarrhea in 3 (13.6%), nausea/vomiting in 3 (13.6%), hypotension in 2 (9.1%) and constipation in 2 (9.1%). Grade III-IV peripheral neuropathy, thrombocytopenia and respiratory infections were observed in 3 (13.6%), 2 (9.1%) and 3 (13.6%) patients respectively. A case of grand mal seizure during bortezomib infusion was noted.*Conclusion*: Bortezomib proved in our study to be an effective regimen in the treatment of relapsed/refractory MM, yielding very high response rates, while toxicity was acceptable. However, EFS was short, though a longer follow-up may be required in order to estimate patients' outcome more accurately.

1413

DECREASED γ- δT CELL RECEPTOR (*TCR GD*) EXPRESSION IN PERIPHERAL BLOOD LYMPHOCYTE POPULATION AND REDUCED SERUM OSTEOPROTEGERIN CONCENTRATION AS A TUMOUR ADVENTAGE MARKERS IN MULTIPLE MYELOMA (MM) PATIENTS

S.E. Sowinska, U.Z.L Usnarska-Zubkiewicz, K.B. Kuchmister, K.K. Kuliczkowski

Wroclaw Medical University,Poland, WROCLAW, Poland

Backgrounds. γ-δ T lymphocytes (γδ T) appear to posses intrinsic cytolytic activity against tumour cells in carcinomas, sarcomas and lymphomas. OPG is known as natural inhibitor of osteoclastogenesis. Aim: determine a mean percentage (%) of γδ T cells in peripheral blood and serum osteoprotegerin (OPG) concentrations of untreated MM patients (pts) and verify the impact of peripheral blood γδ T cells presence and serum OPG levels at the time of diagnosis on MM clinical adventage. *Material and Methods.* 25 newly MM pts, admitted to Department of Hematology, Blood Neoplasms and Bone Marrow Transplantation of Wroclaw Medical University beetwen 2002-2005 were included into analysis. According to Case bone disease staging system: 6 pts were of 0 stage,4 pt of 1, 3 of 2 and 12 of 3 stages. Samples of blood and sera were taken at the time of MM diagnosis. γδ T cells were estimated by flowcytometry (FACS), using a fluorescence-activated cell sorter and monoclonal antibodies (MoAbs: Ab-anti TCRγ1-FITC (Becton-Dickinson), Ab-anti CD14-PE/CD-45-FITC [Leukogate] and CD3-PE. Serum OPG was estimated in enzyme-linked immunoabsorbent assay (ELISA, Biomedica GmbH, Wien, Austria). *Results.* In 12 of MM pts in 3 bone stage (group I) gd T cells percentage, contained in interval 0,5 - 7,2 (mean = 2,75, SD = 1,9) was significantly (p=0,01) decreased as compared with 13 of MM pts in 0+1+2 bone disease stages (group II): 1,4 - 12,4 (mean6,9, SD = 4,75). Despite a lack of statistical significance, the favorable trend was observed that serum osteoprotegerin (OPG) concentrations in MM patients with abbundant bone involvement (group I) fluctuated from 0,9 to 5,3 pmol/ml (mean = 2,34, SD = 1,47) and was also lower than in MM pts with less advanced bone destruction (group I): 1,4 - 7,4 pmol/ml (mean = 3,68, SD = 2,07) (p=0,08). Moreover, possitive corellation between peripheral blood γδ T cell percentage at the time of diagnosis and serum OPG concentration was found: r = 0,48 (p= 0,03).*Conclusions.* In MM decreased γ-δ T cell percentage in peripheral blood and reduced serum osteoprotegerin concentration, measured at the time of diagnosis seems to be advanced tumour markers in clinical practice.

1414

CARDIAC AMYLOID DEPOSITION AS A CAUSE OF MYOCARDIAL DYSFUNCTION IN MULTIPLE MYELOMA

D. Platogiannis,[1] D. Mihou,[2] C.H. Lafaras,[2] E. Mandala,[3] E. Verrou,[2] A. Banti,[2] K. Zervas,[2] T.H. Bischiniotis[2]

[1]*'Theagenion' Cancer Center, THESSALONIKI, Greece;* [2]*'Theagenion' Cancer Center, THESSALONIKI, Greece;* [3]*'Hippokration' General Hospital, THESSALONIKI, Greece*

Backgrounds. Amyloidosis (AL) occurs in 10-20% of patients with multiple myeloma (MM). Around 40% of them manifest cardiac involvement on echocardiogram, in only half of which, overt congestive heart failure is observed at presentation. Doppler echocardiography best assesses myocardial function in AL by demonstrating inflow restriction during diastole. The precursor of B-type natriuretic peptide (pro-BNP) is a specific and sensitive biomarker secreted by the muscle cells of left ventricle (LV) in response to ventricular stress allowing early detection of myocardial dysfunction. *Aim.* To study the correlation between the extent of amyloid infiltration of myocardial wall, as expressed by the thickness of interventricular septum (IVS) and the degree of myocardial dysfunction, as translated by the value of E/A wave ratio on Doppler echocardiogram and the serum levels of pro-BNP. Methods. Twenty-six patients with multiple myeloma and no other medical condition that could affect the thickness of IVS or myocardial function, entered the study and were divided into Groups A and B according to the thickness of IVS (>14mm and ≤14 mm respectively). Eighteen healthy individuals with thickness of IVS <11mm, were used as control Group C. Doppler echocardiography was performed in order to estimate the thickness of IVS and E/A wave ratio and blood was drawn in order to measure pro-BNP serum levels. One-way-ANOVA tests were used to compare the values of E/A wave ratio and the measurements of pro-BNP serum levels between groups. Differences were assessed using the long-rank test. Results. Group A included 15 patients, 10 males and 5 females of medi-

an age 54 years (43-67), Group B 11 patients, 5 males and 6 females of median age 50 years (41-63) and Group C 18 patients, 12 males and 6 females of median age 48 years (39-57). The mean values of E/A wave ratio in Groups A, B and C were 0.7, 0.92 and 1.12 respectively with statistically significant difference ($p<0.01$) among all groups. The mean serum pro-BNP levels in Groups A, B and C were 679 pg/ml, 384 pg/ml and 85 pg/ml respectively, also with statistically significant difference ($p<0.01$) among all groups. Conclusion: Cardiac amyloid deposition in MM patients, is responsible for LV stress and myocardial dysfunction, the degree of which correlates to the extent of myocardial amyloid infiltration. Doppler echocardiography and measurement of pro-BNP levels can be used for early detection of subclinical myocardial damage, before the latter evolves into overt heart failure.

1415

HIGH INCIDENCE OF OSTEONECROSIS OF JAW IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH ZOLEDRONIC ACID

F.A.W. Wandroo,[1] A.S. Shiekh,[1] F.V. Vohra,[1] M.W. Watts,[2] B.R. Ryely[1]

[1]*Sandwelland West Birmingham Hospitals N., BIRMINGHAM, United Kingdom;* [2]*Sandwelland West Birmingham Hospital nh, BIRMINGHAM, United Kingdom*

Background. Bisphosphonates are of proven value in the prevention of skeletal-related events in patients with multiple myeloma and metastatic bone disease. In multiple myeloma these are used routinely in patients with skeletal lytic lesions. Several classes of bisphosphonates are used, however recently the use of zoledronic acid has increased due to its high potency in preventing bone mineral loss. This has however been associated with a new oral complication, osteonecrosis of jaw, in a proportion of patients with myeloma. The pathogenetic mechanism is thought to be bisphosphonate induced increased osteoclast activity and reduced blood flow in the bone which delays bone healing.We evaluated in our institution all patients of multiple myeloma and metastatic cancer who have been treated with intravenous bisphosphonates and looked at the incidence of osteonecrosis of jaw, underlying predisposing factors and outcome after management of these patients. *Aims*. To look for the incidence, clinical presentation, underlying predisposing factors for the development of osteonecrosis of jaw in patients with multiple myeloma and metastatic cancer treated with intravenous bisphosphonates To evaluate the treatment outcome and formulate guidelines for the prevention of this complication. *Methods*. We evaluated all patients of multiple myeloma and metastatic cancer treated in our Institution with intravenous bisphosphonates for the occurrence of osteonecrosis of jaw. We looked at the type of bisphosphonate used, duration of use, concurrent use of chemotherapy, clinical presentation, precipitating factors and the treatment outcome. The suspected patients were evaluated and managed at the oral surgery department. *Results*. We found out of a total of 26 patients with multiple myeloma who were on intravenous bisphosphontes 6 developed pathologically proven osteonecrosis of jaw. Interestingly all six patients were females and all of these had received zoledronic acid. None of the 36 patients with non-haematological cancer who had received intravenous bisphosphonates developed osteonecrosis of jaw. The patient profile of the myeloma patients who developed osteonecrosis is given in table-1.Mean age of patients was 62 years. Median duration of onset of symptoms after the start of zoledronic acid was 45 months (range 24-68). Median number of cycles of bisphosphonate used was 44 monthly cycles (range 24-67). The commonest presentation was jaw pain, three patients had visible facial swelling. Two of the patients had non-healing sockets. Five patients were on concomitant chemotherapy (Thalidomide, cyclophosphamide, melphalan, interferon and steroids). Radiographs did show characteristic lytic lesions of multiple myeloma but as early inflammatory process is difficult to appreciate on x-rays only two patient films showed the typical healing response around the necrotic bone related to osteonecrosis of jaw. Biopsy of all patients was performed, tissues from non healing sockets showed florid lymphoplasmacytic infiltrate, histopathology of the exposed bony patches revealed abundant purulent material and necrotic bone and laboratory findings of the patient having soft swelling in the anterior arch were presence of hyperplastic fibroepithelial growth . Orodental hygiene was poor in all patients. Only two patients had history of dental extraction.None of the patients had history of radiotherapy to head and neck. In all six patients zoledronic acid was stopped and was switched over to sodium clodronate. Debridement of non-healing sockets and exposed areas of bone were also carried out. Ptients were given long term antibiotics and were encouraged towards maintenance of good oral hygiene and regular use of antiseptic mouth washes. These measures brought substantive improvement to the quality of life of patient and relieved there symptoms to a certain extent however the lesions did not heal completely. After noticing these complications we discouraged the use of zoledronic acid outside the context of a clinical trial. *Summary*. Osteonecrosis of jaw following intravenous bisphosphonates has been noticed since 2003. Several case series have been published but as yet no proper guidelines for its prevention have been published. The incidence of jaw necrosis in patients with myeloma in most published series is about 4% Majority of cases occur following zoledronic acid (80%) however pamidronate and even alendronate have been implicated. Dental procedures, poor orodental hygiene, radiotherapy and in some cases concomitant use of anti-angiogeneic agents like thalidomide or corticosteroids are thought to be the commonest predisposing factors. In our short series of cases, we found a high incidence of (6/26) this complication in patients with multiple myeloma. This is worrying for the patients and treating physicians. Large case studies are warranted to delineate the predisposing factors and formulate management guidelines. Results of the U.K. MRC myeloma IX trial with regard to this complication in which patients with myeloma are randomized to receive zoledronic acid or intravenous pamidronate will be interesting We suggest that physicians and dental community should liaise closely with each other in the identification and management of this dreaded complication. We suggest patients patients should be informed of the risk of osteonecrosis. All patients should be reviewed by the oral surgeons before the start of bisphosphonates and any dental infections removed.

Table 1. Showing patients profil.

Pt.No	*Age/Sex*	*Date of diagnosis*	*Medications*	*No of infusions*	*Sign/symptoms*
1	62/F	April 1999	Cyclophosphamide Zoledronate	61	Facial pain/ swelling
2	56/F	April 1995	Cyclophosphamide Zoledronate	67	Jaw pain Non healing sock-
et					
3	43/F	July 2003	Zoledronate	24	Facial pain swelling Non healing sock-
et					
4	68/F	Sep 1998	Thaliodomide steroids Zoledronate	24	Jaw pain
5	68/F	April 1999	Cyclophosphamide Zoledronate	58	Jaw pain Non healing sock-
et					
6	79/F	Sep 1999	Steroids melphalan Cyclophosphamide Zoledronate	28	Facial swelling

1416

CLINICO-BIOLOGICAL PROFILE AT FIRST PRESENTATION OF MULTIPLE MYELOMA PATIENTS. RETROSPECTIVE STUDY

I. Ionita, R. Laza, A. Isac, H. Ionita

UMF Victor Babes Timisoara, TIMISOARA, Romania

Backgrounds. Despite all the clinical experience gained since now, the recognition of the disease in early stage continues to be unsatisfying. Aims. We analised the clinico-biological profile of multiple myeloma (MM) patients at their first presentation in the clinic, in order to identify the stage and the factors which could increase the efficiency of the early diagnosis. Methods. We retrospectively analized a group of 125 patients with monoclonal gamapathy hospitalised in the Hematology Clinic of Timisoara during ianuary 1999 - may 2005. We studied the structure of the analised group, the asimptomatic period in the disease evolution, the nature of the clinical manifestations, biological and paraclinical modifications, the structure of borowed diagnostics, the hospi-

tal units that sent us patients with MM suspicion, the stage of the disease. We had 56% men, 44% women. *Results.* 88% of MM patients, 8% of Waldenstrom disease, 7% of solitary plasmocytoma and 5% of MGUS. Biological modifications were: increased ESR 15%, anemia 15%, hypergamaglobulinemia 8%, hyperproteinemia 1,5%, monoclonal migration 1,5%. The most important clinical sign was the bone sindrom (bone pain and lisis). Bone lisis were localised on the: skull 48%, rib cage 28%, pelvis 18%, lumbar spine 6%. Neurological manifestation were 23% progresive radicular pain and 77% sensory or motor defects. General signs (astenia, disiness, low efort capacity) were present in 88% of the cases and infections in 30% of the cases. The profile of the clinics that contribued to the identification of the disease was: 31% internal medicine, 10% gastroenterotogy, 11% nephrology, 10% neurosurgery, 5% cardiology, 5% dialisis, 5% pneumophtisiology, 3% chest surgery, 3% neurology, 3% urology, 2% reumatology, 2% plastic surgery, 2% general surgery, 2% oncological surgery, 2% oncology, 2% gynecology, 2%ORL. At diagnosis patients were in stage I 11%, stage II 16%, stage III 73%. Borowed diagnosis were: anemia 17%, lumbar disorder 11%, toracic tumor 11%, monoclonal gamapathy 6%, chronic renal failure 6%, radiculonevritis 6%, reumatic disorder 4%, fronto-parietal tumor 4%, colagenosis 2%, artrosis of the cervicodorsolumbar spine 2%, gonartrosis 2%, superior digestive haemorrage 2%, medulary hypoplasia 2%, digestive neoplasia 2%, hepatosplenic sindrom 2%, Raynaud sindrom 2%, prolonged fever sindrom 2%, pulmonary neoplasia 2%. What is realy important in the results of our study is that 74% of the patients with MM suspicion came from other hospital units not from the primary medicine units and also the percent of patients in stage III at diagnosis was 73%. *Conclusions.* 73% of patients were stage III at first presentation which means that there are some problems to recognise and supervise this disease at primary medicine units. There are some biological test that remain unused for the patients which could contribue at early diagnosis of MM.

Funding: Study supported by grant TD 22 no.27690/14.03.2005 of CNCSIS (Romanian National Council of Scientific Research)

1417

SERUM-ASCITES ALBUMIN GRADIENT DIFFERENTIATES TWO TYPES OF ASCITES IN MULTIPLE MYELOMA: REPORT OF TWO CASES AND REVIEW OF THE LITERATURE

T. Sikorski,[1] E. Marcinowska-Suchowierska,[1] J. Grazka,[1] J. Wejman,[2] M. Werczynska,[3] R. Maryniak,[4] M. Prochorec[4]

[1]*Medical Center for Postgraduate Educatio, WARSAW, Poland;* [2]*Dept. of Pathology, W. Orlowski Hospital, WARSAW, Poland;* [3]*Diagnostic Laboratory, Orlowski Hospital, WARSAW, Poland;* [4]*Pathology Dept., Institute of Hematology, WARSAW, Poland*

Ascites is extremely rarely encountered in multiple myeloma (MM), either at presentation, or in the course of the disease. To diagnose myelomatous ascites tumor plasma cells and/or monoclonal protein must be demonstrated in peritoneal fluid. However, a detailed characteristics of myelomatous ascites as a clue to its pathogenesis has not been given. To reveal characteristics of ascitic fluid in myelomatous ascites we reported two cases of MM-related ascites and reviewed the pertinent literature. MM has been diagnosed on the International Myeloma Working Group criteria (*Br. J. Haematol. 2003;121:749-757*) and staged by Durie and Salmon (*Cancer 1975;36:842-854*). Peritoneal fluid samples have been assessed according to Runyon's guidelines (*Hepatology 2004;39:1-16*). We determined ascitic total protein, M protein, and albumin, serum-ascites albumin gradient (SAAG), ascites to serum total protein and lactate dehydrogenase ratios, total nuclear cell count and differential, culture and cytology. A chylous ascites has been confirmed by ascitic triglycerides level > 200 mg/dL. From English and French full text articles searched out by Medline all cases of MM-related ascites with an adequate ascitic fluid data have been chosen for analysis. We assumed that at least ascitic protein, total nuclear cell and/or plasma cell count, and given or calculable SAAG must have been available. The nonparametric Mann-Whitney test has been applied. From January 2004, we managed 2 patients with MM presenting as ascites (a 91-yr-old male with IgA type at IIIA stage and a 72-yr-old female with IgG type at IB stage). In both patients ascites was chylous, had characteristics of an effusion, high SAAG, and contained tumor plasma cells. At first, our patients were treated with weekly dexamethasone, then received melphalan and prednisone course, but survived only 1 and 3 months, respectively, from a diagnosis of ascites. Of 34 cases of MM-related ascites reported during the last 40 years, we could choose only 9 additional patients who had an adequate ascitic fluid characteristics. On the whole, we analyzed 11 cases (Table) and revealed two types of myelomatous ascites with low (< 1.1 g/dL) and high (≥ 1.1 g/dL) SAAG. In the former a median ascitic total nuclear cell count and plasma cell count were higher than in the latter, 4100/μL (range 1000-9000) and 340/μL (range 40-800) (p=0.008), and 3031/μL (range 1000-8550) and 120/μL (range 30-450) (p=0.016), respectively. Three of 11 cases had a chylous ascites.

Table 1.

First author/ Year	*Age (yrs) sex*	*Ig Type*	*Ascitic fluid characteristics*					
			Color	*M band (g/dL)*	*Protein (g/dL)*	*SAAG (g/dL)*	*Cell /μL*	*Plasma cells/μL*
Poth/1971	45/F	Gk	bloody	NA	6.4	0.4	9000	8550
Thomas/1973	NA	NA	NA	NA	4.5	>1.1	150	30
Higby/1975	76/M	Gk	chylous	2.5	5.9	1.0	NA	1000
Greer/1985	57/M	Gk	bloody	4.6	7.1	<1.1	4100	1640
Gorg/1988	44/M	Ak	bloody	1.5	5.8	<1.1	1000	+
Alegre/1999	51/F	Gk	yellow	+	2.4	>1.1	40	37
Keren/1999	71/F	λ	yellow	+	2.9	0.9	6600	4422
Singh/2005	45/M	Gλ	NA	+	2.0	>1.1	340	136
Inoue/2005	51/F	Gλ	yellow	1.8	>2.9	0.8	2220	+
Sikorski/2006	91/M	A	chylous	3.2	6.9	1.7	600	450
Sikorski/2006	72/F	Gk	chylous	1.3	3.6	1.6	800	120

NA, not available data; F, female; M, male.

A median survival from ascites development was the same and very short regardless of ascites type and averaged 3.5 and 4.25 months, respectively. In MM two types of myelomatous ascites may be encountered. A low SAAG type with high cell counts is secondary to peritoneal involvement by MM with plasma cells proliferation or homing in the peritoneum. A high SAAG type with low cell counts is an example of mixed ascites with a myelomatous liver infiltration leading to portal hypertension as an additional pathogenetic factor. In both types an overlapping of failed lymphatic drainage may brings about a chylous ascites.

1418

INCIDENCE AND PRESENTATION OF OSTEONECROSIS OF THE JAWS IN MULTIPLE MYELOMA PATIENTS TREATED WITH BISPHOSPHONATES

C. Lalayanni, N. Neokleous, D. Sotiropoulos, A. Papalexandri, R. Saloum, A. Anagnostopoulos, A. Fassas

G. Papanicolaou Hospital, THESSALONIKI, Greece

The administration of bisphosphonates is currently a mainstay in the management of hypercalcemia and bone absorption in a setting of malignancies, such as multiple myeloma or solid tumors. The main side effects of bisphosphonates are renal dysfunction, influenza-like syndrome and anemia. Osteonecrosis of the jaws (ONJ) is a recently reported complication of bisphosphonate use, especially intravenous formulations containing nitrogen. The unique properties of the oral cavity (bacterial flora, frequent injuries) combined with the antiangiogenic properties of bisphosphonates potentially contribute to the development of ischemic lesions in the jaws. The incidence of ONJ in MM patients treated with bisphosphonates is largely unknown as the diagnosis is usually made by oral and maxillofacial surgeons. We report six patients, 2 women and 4 men, (median age 65 years) with bisphosphonate-associated osteonecrosis of jaws. MM therpay consisted of melphalan-prednisolone (4/6 patients), VAD-Caelyx or Thalidomide (one patient each). Four out of six affected patients were treated long-term (29 to 46 months) with either pamintronate or zolendronic acid at monthly intervals; two patients received zolendronic acid for, respectively, 9 and 11 months only. Over the same five-year period, a total of 106 patients with MM were treated with bisphosphonates in our department. Therefore, the incidence of ONJ in our MM series was 6% (6/106 cases). Osteonecrosis typically presented with a painful exposed necrotic bone at the site of previous dental extraction (5 patients) or injury (1 patient). Biopsies of the jaw lesions showed no evidence of malignancy, while the cultures revealed normal oral flora. The lesions were not managed easily: most patients required surgical excision despite broad-spectrum antibiotic therapy and bisphosphonate discontinuation. One patient responded to hyperbaric oxygen therapy. The two patients who received bisphosphonates for less than a year showed a better response to therapy. In conclusion, osteonecrosis of the jaws is a serious complication of bisphosphonates which

requires alertness and prompt management. Rigorous oral hygiene and avoiding extensive dental procedures in patients who receive bisphosphonates could assist in preventing this complication.

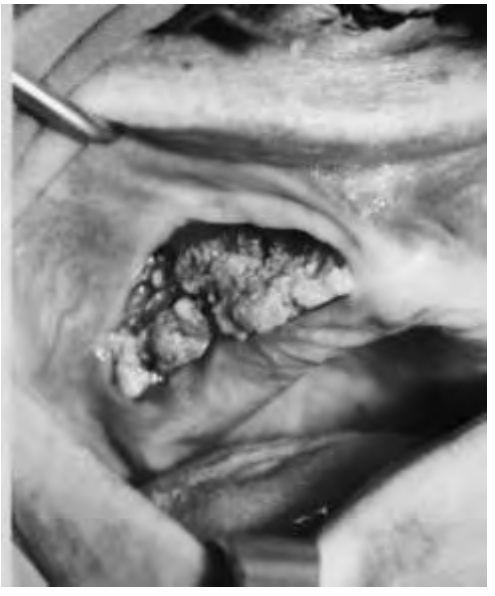


Figure 1. ONJ in a bisphosphonate-treated MM patient.

1419

OSTEONECROSIS OF THE JAW IN MULTIPLE MYELOMA PATIENTS: MONOCENTRIC EXPERIENCE

A.M. Liberati,[1] G. Braccalenti,[1] A. Rauco,[1] L. Falchi,[1] G. Desantis,[1] A. Lugini,[2] P. Cerroni[1]

[1]*Medicina Interna e Scienze Oncologiche, PERUGIA, Italy;* [2]*Pr General Hospital S. Camillo De Lellis, RIETI, Italy*

Multiple myeloma (MM) is a common lymphoproliferative disorder. Modern therapies have remarkably improved the survival of affected patients. Thus, long term adverse events related to chemotherapy and/or ancillary treatments are observed with increasing frequency. Bisphosphonates (BP) are synthetic analogues of pyrophosphate. These compounds have been approved for treatment of cancer-related hypercalcemia and bone lytic involvement by MM and solid tumors. Zoledronic acid (ZA) is the most potent BP. It has antiangiogenetic activity and inhibits osteoclastic differentiation and normal bone turnover. Recently, several cases have been reported of avascular osteonecrosis of the jaw (ONJ) associated with the use of ZA in patients with MM. Jaw is the only bone exposed to the outside and often site of traumatisms which induce osteoclasts activation. The objective of this monocentric analysis consists in the retrospective evaluation of the effects of treatment duration with ZA on the onset of ONJ in a cohort of 64 patients which were autotransplanted at our Institution. ONJ was evaluated by craniofacial complex CT and/or MRI followed by tissue biopsy for pathological and microbiological exams. Among the 64 analyzed patients, 5 (3 in long lasting complete remission and 2 in very good partial remission) developed ONJ. Two of this also showed a massive necrosis of both maxillary sinuses. All of the patients referred pain and swelling, two of them also referred purulent discharge and necrotic jawbone exposure. ONJ was documented by biopsy in three of the five patients (3 men and 2 women). BP therapy was discontinuated in all cases. Three patients underwent surgical curettage and all five were treated with antibiotic therapy. The outcome has been resolution of necrosis in three patients, persistence of bone exposure in one patient and oral antral communication and cutaneous fistula in the other. Time to jaw osteonecrosis diagnosis since the beginning of BP treatment was three years in one patient, 4 years in two patients and 7 years in the last two. Osteonecrosis of the jaw in patients with MM can be associated with BP therapy. BP mechanism of action, that includes osteoclasts apoptosis and antiangiogenetic effect, is responsible for reduction of local blood flow and retard in bone repair. This leads to jaw bone damage. Duration of therapy with ZA is crucial in the development of this complication in affected patients.

1420

REMOVAL OF SERUM FREE LIGHT CHAINS BY HEMODIALYSIS IN PATIENTS WITH MULTIPLE MYELOMA

C. Hutchison,[1] P. Cockwell,[1] S. Reid,[2] K. Chandler,[2] G. Mead,[2] A. Bradwell[3]

[1]*University Hospital Birmingham, BIRMINGHAM, United Kingdom;* [2]*The Binding Site Ltd, BIRMINGHAM, United Kingdom;* [3]*Universit of Birmingham, BIRMINGHAM, United Kingdom*

Renal failure is a common complication of multiple myeloma (MM) and is associated with poor outcome. The cast nephropathy which results from the excess free light chain (FLC) production is the main cause of renal failure in MM. There is intense interest in whether rapid normalisation of serum FLC by plasma exchange (PE) and/or haemodialysis can improve renal outcomes. We have previously demonstrated that haemodialysis (HD) using high flux membranes removes a proportion of serum FLC in a non-MM, chronic HD population. However, in the MM setting, where serum FLC levels can be up to 10000 fold higher than normal, HD using standard high flux membranes was less effective. The purpose of this study was to demonstrate the ability of HD with a novel dialysis membrane to remove FLC in patients with MM. Five patients with MM and dialysis-dependent acute renal failure underwent dialysis using a Gambro Protein Permeable HCO 1100 Polyamide membrane (Gambro, Germany). Blood samples before and after each dialysis and samples of dialysate fluid were taken. Patients 1 to 5 had 5, 2,6,10 and 1 dialysis sessions for a mean time of 6, 4, 3, 4 and 6 hours respectively. FLC measurements were performed using the nephelometric immunoassay FREELITE(™) (The Binding Site). To estimate the total amount of FLC removed, the following calculation was applied: mean FLC concentration in dialysate (mg/L) x dialysate volume (L). All 5 patients showed abnormal sFLC levels at the beginning of the study (3 with elevated serum free λ 'patients 1 to 3), 2 with serum free kappa, patients 4 and 5). Dialysis using the Gambro HC1100 membrane was able to reduce sFLC levels in every session, for each patient. Although the amounts of FLC removed from the blood differ depending on the starting level, the mean percentage falls of light chain were 59.6%, 58.6% and 23.7% for the lambda patients and 45.9% and 61.8% for the kappa patients (Table 1). The means of the total amount of FLC removed per dialysis session were 33g, 31g, 8g, 20g and 4g for patients 1 to 5 respectively. We have conclusively demonstrated that HD can remove large quantities of FLC in the context of MM with the Gambro HC1100 dialysis membrane. Given that the total amount of FLC found in the dialysate exceeded the available FLC in the blood, these data suggest that FLC were also removed from the extravascular compartment. Serum FLC are known uremic toxins and contribute to worsening renal function and their rapid removal may be beneficial. The use of extended HD could further improve serum FLC removal in these patients.

Table 1. Table of FLC levels in serum and dialysis.

Patient	*Dialysis*	*FLC type*	*Mean blood pre (mg/L)*	*Mean blood post (mg/L)*	*Mean% Removed*	*Total in dyasilate fluid (g)*
1	5	λ	10626	4310	59.6	33
2	2	λ	9155	3760	58.6	31
3	6	λ	3362	2445	23.7	8
4	10	κ	2758	1489	45.9	4
5	1	κ	861	329	61.8	19.6

1421

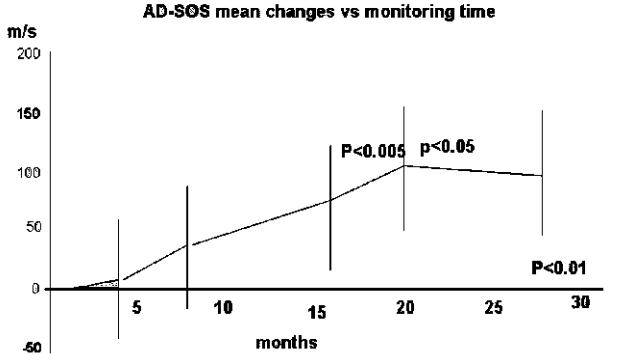
QUANTITATIVE BONE ULTRASONOGRAPHY AT PHALANGES IN PATIENTS WITH PLASMA CELL DISCRASIAS AND ZOMETA TREATMENT

F. Iuliano[1], I. Guzzo[2], A.R. Frascogna[2], F. Deterlizi[2], P. Petitto[3], A. Russo[3], A. Peta[4], M. Baserga[2]

[1]*Azienda Ospedaliera 'Pugliese-Ciaccio', CATANZARO, Italy;* [2]*Cattedra di Pediatria Univ.Magna Graecia, CATANZARO, Italy;* [3]*Azienda Ospedaliera 'Pugliese-Ciaccio', CATANZARO, Italy;* [4]*AZIENDA OSPEDALIERA 'PUGLIESE-CIACCIO', CATANZARO, Italy*

The current aims of bisphosphonates for metastatic bone disease are to prevent skeletal-related events (SREs), reduce bone pain and improve quality of life. Zoledronic acid (Zometa™) is the most potent tested bis-

phosphonate Additional studies are needed to determine the optimal timing, schedule, and duration of treatment in myeloma patients . DBM Sonic Bone Profiler is the only ultrasound device that applies the method of signal analysis in transmission through phalanges. This technique of clinical investigation has proven to be particularly effective in post-menopausal osteoporosis for screening, diagnosis and therapies monitoring To assess Bone Mineral Density (BMD) in patients with plasma cell discrasias along the time of Zometa™ treatment using quantitative ultrasound analysis in transmission through phalanges. 12 pts with MGUS (8 IgGk,4 IgG λ) and 4 pts with myeloma IIIA (3 IgG κ,1 IgA lambda),M/F:6/10 , median age 69 yrs received zoledronic acid 4 mg intravenously every 4 weeks for at least 12 courses. All subjects took daily oral supplements containing elemental calcium (1 g) and vitamin D (400 IU).Myeloma patients also received chemotherapy. Measurements of bone mineral density were performed by ultrasonography at phalanges at baseline and at 1, 3, 6, 9,12,15 and 18 months The ultrasound signal is analysed to generate the test report parameters, AD-SoS (Amplitude Dependent Speed of Sound),UBPI (Ultrasound Bone Profile Index), BTT (Bone Transmission Time) that express the properties of density and structure of bone tissue. As showed in the picture all patients improved AD-SOS togheter with all measured parameters (UBPI and PTT) starting from 4 to 16 month (p<0.005) in comparison with baseline values. Densitometric-structural evaluation of bone tissue at distal metaphysis of the first phalanx of the II, III, IV and V hand finger is safe and useful to evaluate bone quality change during Zometa treatment.



1422

WISKOTT-ALDRICH SYNDROME SHOULD MEDIUM PLAQUETARY VOLUME (MPV) MATTER?

P. Matthioli Luis,[1] H. Ramos,[2] T. Almeida,[3] M.O. Freitas,[3] L. Rosado[3]

[1]*Hospital de Santa Maria, LISBOA, Portugal;* [2]*Hospital do Esprito Santo, VORA, Portugal;* [3]*Hospital Dona Estefnia, LISBOA, Portugal*

Background and Aims. Wiskott-Aldrich syndrome (WAS) is typically X-linked and is characterized by a clinical triad: eczema, thrombocytopenia with small platelets and immunodeficiency (predisposing to autoimmune phenomenon, lymphoproliferative disease and neoplasia). However, there can be polymorphism on several levels *transmission autossomic dominant, transcription cis or trans of the mutated gene* that translate in different clinical evolution. We present a case of very early immunodeficiency, with a diagnostic being less obvious by the fact that the platelets always showed a normal MPV. *Methods/Clinical Case*: A caucasian male infant 1month and 3 weeks old, without gestational or labour problems, is hospitalized with bloody diarrhoea, petechiae on abdominal wall and mild squamous dermatitis. He presented Hb 8,5 g/dL, VGM 87,7fL, reticulocytes 7,9%, leukocytes 13600/mm^3 (atypical lymphocytes 10%), platelets 23000/mm^3, LDH 1093. The marrow smear hasn't revealed maturative alterations on myeloid and erithroid series; lymphoid series -15% of small lymphocytes with high reason nucleus/cytoplasm; megacaryocytes diminished in number and in size with hypolobulation of nuclei. The objective examination showed moderate pallor of skin and mucous membranes, peculiar facies (frontal lumps, short neck), dry skin with dermatitis (maculo-papular erithema) in the neck and retro auricular regions; liver 4 cm beyond costal grid, surpassing medium line; spleen 6 cm beyond costal grid. The diagnostic hypothesis were WAS, congenital or neonatal infection (TORSCH) and auto-immune disease. Gradually a rise is seen in all liver enzymes; the thrombocytopenia floats between 20 and 50.000/mm^3 with MPV between 9 and 11fL and PDW of 80-100%.With further exams, autoimmune disease and TORSCH infection are discarded; another remote hypothesis, Langerhans hystiocytosis was also discarded by the skin biopsy (compatible with atopic dermatitis). Clinical evolution between 3 and 7 months - 3 suppurated acute otitis, 2 gastro-enteritis and an interstitial pneumonia with severe hypoxemia (admittance in intensive care unit). The eczema has now a hemorrhagic component, with blood crusts, spreading from the face, neck, axila, dorsum and inguinal regions; hepato-splenomegaly has grown almost to iliac crest. He is medicated with co-trimoxazole and azithromicin (prophylaxis), folic acid and monthly palivizumab. It was done a complete immunologic study at 6/7 months old, that revealed: hyperγglobulinemia with hyperIgM (without deficit of IgG or IgA); lymphopenia T CD8$^+$; elevated expression of activation markers on T lymphocytes, however without proliferation after in vitro stimulation; NK lymphocytosis. Clearly having evidence of a primary or secondary T immunodeficiency, it is made a search for mutations *ZAP-70* and WASP. At 8 months old he is hospitalized by undetermined feverish syndrome and develops a very severe auto-immune hemolysis with shock that lead to his death. The confirmation arrives a mutation in exon 10 of WASP gene. *Conclusions.* In a case with an immunodeficiency this severe, briefly one has to think in bone marrow transplantation (the family was already being HLA typed). WASP protein seems to be involved in mechanisms of signal transduction between surface receptors and cytoskeleton, provoking defects of chemotaxis, fagocytosis and presentation of antigens to T cells with inappropriate response. In platelets, there are diminished surface glycoproteins and adhesion defects. In literature, we find more and more heterogeneous cases, whether in genetic/phenotypic expression or clinical expression (as platelets with normal MPV), that can help achieving a better understanding upon mechanisms of WAS.

1423

THROMBOTIC THROMBOCYTOPENIC PURPURA: REPORT OF 27 CASES

V. Sotirova, O. Karanfilski, A. Latifi, B. Pavkovic, P. Stojanovic

Clinical Center, SKOPJE, Macedonia

Thrombotic thrombocytopenic purpura (TTP) is a severe multisystemic disorder characterized by microangiopathic hemolytic anemia, thrombocytopenia, fever, fluctuating neurological symptoms and impaired renal function. *Aims*. To present the experience of the Department of Hematology, Clinical Center, Skopje, regarding this issue. Patients and Methods. our study includes 27 patients (pts) with TTP between 1986 and 2006. All patients had microangiopathic hemolytic anemia and thrombocytopenia. There were 14 women and 13 men; the median age was 36 years (range 20-71). It doesn't appear to be related to other diseases. *Results.* the mean period between the first symptoms and the diagnosis was 8,5 days (range 1-21). Neurological symptoms were present in 21 patient, bleeding in 19, fever in 6 and renal impairment in 12pts. Median hemoglobin was 68 g/L (range 41-93); median platelet count was 49x10 μ/L (range 6-130); median reticulocytosis was 6% (range 1-25). Results of screening tests of coagulation showed elevation of FDP in 7 pts. Serum LDH was increased in all patients - median 1748 IU (range 618-4520). Treatment included : corticosteroids in all patients, exchange plasmapheresis (EP) in 12 pts, only plasma infusions in 13 pts, antiplatelet agents in 10 pts. Plasma exchange is currently not available in our country. In one patient with exacerbation during the first TTP episode treatment with Vincristine was introduced. There were 7 complete responders (5 on EP) and 14 deaths (3 on EP). Among the survivors 6 pts relapsed (2 pts had 2 relapses), 2 of them died during the first relapse. The median time delay from the onset of symptoms and treatment initiation lasted for 8,5 days (range 1-21), indicating poor disease recognition. The median time delay from diagnosis to EP was 5,5 days (1-11) suggesting relatively good EP availability. The median treatment duration in all patients was 15,5 days (range 1-40). The median number of EP cycles needed for the platelet stabilization was 4 (range 2-10). *Conclusion*. TTP is a severe disorder necessitating early recognition and diagnosis which would lead to treatment with EP on time. EP improves survival dramatically.

1424

A CORRELATION STUDY BETWEEN SERUM POTASSIUM LEVEL AND AETIOLOGY OF THROMBOCYTOSIS.

L. Ong

Ulster Hospital Dundonald, BELFAST, United Kingdom

Pseudohyperkalaemia is a rarely encountered event among patients with thrombocytosis. Typically, a raised serum potassium level is observed in the absence of renal failure. Occasionally, samples are reported to be haemolysed and results discounted. The interpretation of hyperkalaemia in the presence of renal failure (raised urea & creatinine) is problematic and causes anxiety among clinicians signing the results. The association between pseudohyperkalemia and reactive causes of thrombocytosis is unclear. A five-year audit was conducted on all patients with thrombocytosis who were referred to the Haematology Department in Ulster Hospital Dundonald. Seventy-one patients were identified from the ward registry. Clinical and laboratory data was obtained following chart review and entered into SPSS version 10 software package. A predominantly elderly patient population is treated in this district general hospital. Twenty percent of patients had primary thrombocytosis; two thirds were essential thrombocythemia. The most frequent causes of secondary thrombocytosis were iron deficiency, infection, malignancy and chronic inflammatory diseases. Raised serum potassium was more likely to be noted when platelet counts exceed 800 in myeloproliferative disorders especially primary thrombocythaemia; it was rare among patients with reactive causes of thrombocytosis. A weak correlation was observed between platelet count and serum potassium levels. Pseudohyperkalemia led to patient admission to hospital, administration of calcium resornium, 5% dextrose / insulin, and introduction of low-potassium diet in several patients in an attempt to lower serum potassium level. Failure to correct pseudo-hyperkalaemia resulted in serial venepunctures for U+Es and repeated dosing of potassium-lowering measures. All cases of pseudo-hyperkalaemia were detected by haematologists consulted regarding the aetiology of thrombocytosis. The falsely raised potassium level is due to release of intracellular potassium from the platelets during formation of clot in the 'gel' clotted specimen. This is a time-dependent phenomenon; the use of plasma sample in either Li-heparin or Na-heparin bottles will circumvent this phenomenon.

1425

EFFECT OF VASOPRESSIN AND IT'S ANALOG DGAVP ON PLATELET AGGREGATION

M. Grigorjeva,[1] M. Golubeva,[1] S. Gorbunova[2]

[1]*Moscow State University, MOSCOW, Russian Federation;* [2]*Res. Inst. of pediatric Hematology, MOSCOW, Russian Federation*

It is well known that neurohypophysial peptide vasopressin (AVP) causes blood coagulation due to increased secretion of tissue thromboplastin and factor VIII:C into blood. Besides AVP exerts significant influence on platelet aggregation through interaction with specific receptors. AVP synthetic analog desglycilamide-arginine vasopressin (DGAVP) lacks peripheral hormonal activity and evokes blood procoagulant activity too. The aim of this study was to compare effect of AVP and it's analog DGAVP as inductor of man or rat platelet aggregation. Effect of AVP and DGAVP as inductor of platelet aggregation was studied on platelet-rich blood plasma (PRP) in the children without bleeding disorders or in experiment on white rats. Platelet aggregation was induced by adding the peptides (in final concentration 10-5 M and 10-6 M) to PRP. Our results demonstrate that as AVP as it's analog DGAVP induced platelet aggregation in children PRP and effect of DGAVP was more intensive than AVP effect (degree of aggregation was 43% and 55,5% and rate of aggregation - 13,5% and 20% accordingly). But in experiment on rat we showed that these peptides induced more weak platelet aggregation (degree of aggregation was only 14% - 16,5%). However in this case DGAVP administration lead to more intensive platelet aggregation too. Besides DGAVP lead to reestablishment of platelet ADF-aggregation in children who had got platelet aggregation disorders with ADF. Thus we conclude that synthetic AVP analog DGAVP as natural peptide AVP induces platelet aggregation in man or rat PRP but it's effect is more intensive. Besides our results demonstrate that as DGAVP as AVP effect on platelet aggregation can be both indirect and direct.

1426

TREATMENT OF IMUNE THROMBOCYTOPENIC PURPURA. POSTSPLENECTOMY LATE RESPONSE

C. Ionita,[1] I. Ionita,[1] R. Pacurar,[2] D. Nicola,[2] M. Iordache,[1] H. Ionita[1]

[1]*UMF Victor Babes, TIMISOARA, Romania;* [2]*County Hospital, TIMISOARA, Romania*

Backgrounds. Imune thrombocytopenic purpura (ITP) is a chronic disease with a good response to corticosteroids which is used as first line therapy. Some times after steroid therapy patients relaps and in this case splenectomy in the second line of therapy. *Aims.* We tryed to evaluate the therapeutical results in a group of patients with ITP and the duration of their remision after splenectomy. *Methods.* From may 1990 - may 2000 were hospitalised and treated in the Hematology Clinic 135 patients with ITP. Median age was 48 years with a distribution on sexes: 46 males and 89 females. Our patients 39% presented gastrointestinal bleedings, 52% had sclerotegumentary bleedings and 9% had bleedings in the central nervous system. The most of the patients 91% were treated with corticosteroids, 12% received steroids and imunoglobulins. 35% (47 patients) had a splenectomy because they relapsed after steroids or they needed very high doses of corticosteroids for a safe number of trombocytes. From those 47 patients with splenectomy 19 were males and 28 were females with a median age at the time of splenectomy of 38 years. The medium time from diagnosis to splenectomy was 3,5 years (0,6-96 months) The response to splenectomy was defined as followes: complete response (CR) a number of trombocytes higher than 150.000/mms for more than 4 weeks, partial response (PR) trombocytes between 50.000-150.000/mms lasting more than 4 weeks and relapse a number of trombocytes under 50.000/mms. *Results.* The medium follow-up time was 7 years (2-10 years). The overall response was 79% with 58% of CR and 21% PR. From 47 patients with splenectomy 15 patients relapsed and 5 of this 15 were in CR after steroid therapy following splenectomy. The long therm follow-up in CR and PR proves a good, stabil and durabil response in time for more than 7 years. Post splenectomy complications in the study group were not significant. *Conclusions.* Our study proves that patients with chronic imune thrombocytopenic purpura who failled corticotherapy get a safe and durable response in time after splenectomy.

1427

INADEQUATE RESPONSE TO RITUXIMAB IN PATIENTS WITH CHRONIC REFRACTORY IMMUNE THROMBOCYTOPENIA (ITP)

S. Mitra, R. O'Donnell, P.T. Murphy

Beaumont Hospital, DUBLIN, Ireland

Background. Immune Thrombocytopenia is an autoimmune disease which involves opsonisation of platelets by auto-antibodies directed against different surface glycoproteins, leading to their premature destruction , in the reticulo-endothelial system. Recent studies suggests that Rituximab,a chimeric monoclonal antibody (against CD 20^+ B cells) is useful in the treatment of chronic refractory ITP.However, in comparison,at our centre,the response to Rituximab has been poor. *Aims*. Retrospective review of platelet counts(over a period of 6 months) of 4 patients treated with Rituximab.

Table 1. Rutuximab in Chronic Refractory ITP.

Patients	*Age Sex*	*Co-morbidity*	*Treatment Pre Rituximab*	*Platelet Count Pre-Rituximab*	*Platelet Count Post Rituximab*	*Treatment Dring & After Rituximab*	*response*	*Tome Maximum Response*
1	35 yr/F	Nil	Ig Steroids	36	40	Ig Anti-D	Minimal Response	4 wk
2	50 yr/M	NIDDM Hypercholest-erolaemia IHD TIAs	Ig Steroids	69	70	Nil	No Response	Not Apllicable (NA)
3	68 yr/F	Osteoarthritis	Steroids Ig Anti-D Danazol	34	20	Ig	No Response	NA
4	80 yr/F	-	Ig Steroids	29	28	Splenectomy	No Response	NA

Methods. We have used 4 cycles of Rituximab (375 mg/m^2) administered at weekly intervals to 4 patients (3 females,1 male;mean age 58 years) with chronic refractory ITP. All 4 patients had previous treatment with Immunoglobulins.Three patients had had prior treatment with Prednisolone; one did not receive Prednisolone because he was a Diabetic.One out the four patients had also received prior treatment with Anti D and Danazol (along with Immunoglobulins and steroids).

Response Criteria: A Complete Response(CR) was defined as a rise in platelet count >100×10⁹/L,a Partial Response(PR) as a rise in Platelet count >50×10⁹/L and a minor response(MR) as a rise in Platelet count <50×10⁹/L. No Response(NR)was defined as no increase in Plateletcount. *Results.* Of the 4 patients 1 had a Minor Response and 3 had No Response. However Rituximab was well tolerated in all 4 cases with no major side-effects. *Conclusions.* Our results suggest that Rituximab hardly made any impact on the platelet count of these 4 patients with chronic refractory ITP. Previous studies of Rituximab in ITP has shown an overall response rate of around 50%.However such initial results must be considered in the light of positive report bias,small numbers, lack of long term follow up and lack of randomised controlled trials.In addition, data on short and long term side-effects of Rituximab are lacking. Thus, Rituximab is an unproven treatment for chronicrefractory ITP. Perhaps novel agents like Thrombopoeitin Receptor Agonists should be considered for these patients with chronic ITP,in the setting of a Clinical Trial.

1428

EFFECTS OF VARIOUS THERAPEUTIC REGIMENS ON PLATELET FUNCTIONS IN PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

M. Akay, E. Akin, Z. Gulbas

Eskisehir Osmangazi University Hospital, ESKISEHIR, Turkey

Bleeding and thrombosis are common causes of morbidity and mortality in patients with myeloproliferative disorders (MPD). Qualitative platelet abnormalities are frequently found in these patients and range from platelet hypofunction, acquired storage pool disease and/or platelet membrane defects, to abnormalities suggesting increased platelet reactivity, increased plasma β thromboglobulin levels or shortened platelet survival. In the present study, we aimed to investigate platelet function abnormalities using both optical platelet aggregometry and whole blood platelet aggregometry and evaluate the effects of various therapeutic regimes on these abnormalities, in patients with MPD. 45 patients with newly diagnosed chronic myeloproliferative disorders (26 CML, 11 PCRV, 8 ET) were enrolled. Median age was 54; there were 23 females and 22 males. At the study entry, whole blood count, PT, aPTT, fibrinogen, platelet aggregation studies by luminesance method in whole blood and by optical method in PRP, ristocetin cofactor activity were performed. The agonists used were; ADP, Arachidonic acid (AA), Ristocetin and Collagen. Platelets were considered to be hyperactive if at least one result (aggregation or ATP release with one agonist) was above the reference range, and hyporactive if at least one result (aggregation or ATP release with one agonist) was below the reference range. Mixed hypo- and hyperactive platelets were considered present when at least one result (aggregation or ATP release) was below and above the reference range, respectively. Repeat platelet function studies were performed in 20 patients, following specific therapy regimes. By luminesance method; before therapy 15/45 patients had platelet hyperfunction, 17/45 patients had coexistence of hyper- and hypofunction and 12/45 patients had platelet hypofunction. 1/45 patient had a normal result. After therapy 13/20 patients had platelet hypofunction, 2/20 patients had platelet hyperfunction, 2/20 patients had coexistence of hyper- and hypofunction while 3/20 patients had normal results. By optical method; before therapy 18/45 patients had platelet hypofunction, 9/45 patients had platelet hyper- and hypofunction, 7/45 patients had platelet hyperfunction whilst 11 had normal results. After therapy 15/20 patients had coexistence of hyper- and hypofunction, 4/20 patients had platelet hyperfunction, 1/20 patients had platelet hypofunction while none of the patients had normal results. We conclude that; 1. Different platelet function defects are observed in most of patients with MPD 2. Patients with CML have platelet hypoaggregability while patients with PCRV and ET have platelet hypoaggregability. 3. Our observations highlight the need to use WBPA to select patients for antiplatelet therapy in MPD. 4. Luminesance method appears to be more sensitive than optical method to evaluate platelet functions.

1429

ADIPONECTIN ADDED INTO THE PLASMA OF HEALTHY PROBANDS DOES NOT AFFECT PLATELET AGGREGABILITY

J. Proskova, D. Stejskal

Sternberk Hospital, STERNBERK, Czech Republic

Background. Adiponectin exhibits important antidiabetic and antiatherogenic effects. Although hypoadiponectinemia is associated with obesity-related metabolic and vascular diseases, the role of adiponectin in thrombosis remains elusive. Recent paper informed that adiponectin deficiency in adiponectin knockout male mice leads to enhanced trombus formation and platelet aggregation. *Aims*. Evaluace of added adiponectin effect into the plasma in platelet aggregability. *Methods*. 6 healthy nonobese healthy probands were tested. In all of them platelet aggregability and adiponectin values were measured. Human adiponectin (Biovendor; Czech Republic) was added to PRP in different concentrations (100; 75; 50 and 25 ng/l). Than PRP was 5 min incubated and was evaluated induced platelet aggregation using CPG (Analytical Control Systems) at 3 μmol/l as the final concentration of CPG added to PRP with an Apact II platelet aggregometer (Labitec GmbH). Induced aggregation extent was defined by the slope of aggregation curve. *Results.* Adiponectin values had normal distibution in tested group (13,7-15,8 ng/l). Neither of tested probands had significat difference of the slope CPG values, even if 100 ng/l adiponectin concentration was added. *Conclusions*. The present study did not verify hypothesis about the in vitro human hyperadiponectinemia as an antithrombotic factor. Adiponectin concentration about 10 ng/l have similar antithrombotic *in vitro* action as values upper 100 ng/l.

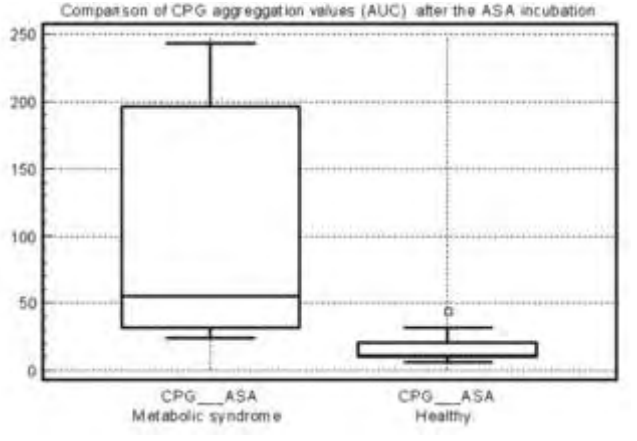
1430

PRESENTATION OF NEW METHOD FOR ASA RESISTANCE DETECTION

J. Proskova, D. Stejskal

Sternberk Hospital, STERNBERK, Czech Republic

Backgrounds. aspirin resistance seems to be an important prognostic factor in patients with coronary artery disease, but there is limited data on its correlation to clinical outcomes. Various methods for both in vivo and in vitro platelet function exist. In late 1990s, a novel in vitro inductor of platelet aggregation - cationic propyl gallate (CPG) was introduced into clinical practice, announced as an *unprecedented*, highly sensitive and specific method for assessment of aspirin resistence. In classic aggregometry problem with patients compliance remain unresolved. Recently there were present information about chance for ASA resistance testing by virtue of in vitro aggregation test with ASA addition. *Aims.* evaluate platelet ASA resistance with platelet CPG aggregation after ASA addition.



Methods. 20 healthy individuals and 20 patients with metabolic syndrome were evaluated. No individuals were ASA treated. In all of probands was performed platelet aggegometry (Multiplate) after CPG induction. In part of whole blood was supplement solution of ASA (Aspisol, Bayer) and was perform aggregometry, over again. *Results.* healthy probands have higher difference between AUC before and after the ASA pretreatment. (p<0,01, Kruskal Wallis) than probands with metabolic syndrome. CPG have higher difference before collagen (p< 0,05). AUC of aggregometry line in all of healthy probands had significant reaction after Aspisol addition. On the contrary, AUC of patiens with metabolic syndrome reacted different. *Conclusion*: authors presented frequent ASA resistance existence in individuals with metabolic syndrome against healthy. At the same time presented new ijn vitro method for ASA resistance detection which eliminace patient non compliance errors.

1431

ASPIRIN STRENGTHENS ANTITHROMBOTIC EFFECT OF HEPARIN-LIKE ANTICOAGULANT FROM PLANT

Y.U. Lyapin, Y.U. Obergan

Moscow State University, MOSCOW, Russian Federation

Background. Effective antithrombotic agents possessing antiplatelet activity, are preparations such as aspirin, dipiridamol and others. Low molecular weight heparin also shows antithrombotic activity. Antithrombotic effect of plant heparin-like anticoagulant from roots of a peony at chronic intranasal administration has been shown. *Aims*. The purpose of the present work consist in studying antithrombotic effects plant heparin (PH) from Paeonia suffruticosa with antiplatelet aspirin at simultaneous peroral application of these preparations. *Methods*. In the work methods electrophoretic, chromatographic and spectral analysis were used. The formation of blood clots was carried out on method Wessler with our updating - in isolated by metal clips a fragment vein jugular entered thrombin with activity 3-5 sec (thrombin+stas of vessel). PH (1mg/mL) and aspirin (1 mg/mL) mixed in the ratio 1 : 1 (w/w). This mix was administrated peroral to animals (albino rats) in daily volume 0.5 ml/kg of body weight within 7 days before formation of blood clots. Antithrombotic effect was estimated on frequence of cases of thrombus formation and on weight of blood clots after 1 hour after thrombus formation. *Results*. At formation of blood clots of animals on a background of action PH + aspirin thrombus either were not formed (did not come to light) or were small (in case of their formation). So, quantity of cases of thrombus formation in experiment on a background administrated PH + aspirin made 8 per cent, on a background administrated one PH made 58 per cent from the control = 100 per cent (administration of 0.85% solution of NaCl). The average weight of the formed blood clots in experiment with PH + aspirin made only 4-6 per cent and with PH - 15-16 per cent from the control (100 per cent). Furthermore it has not been marked any collateral negative influences PH + aspirin and one PH (for example, haemorrhagic action). Conclusions. Administration aspirin together with PH has shown higher antithrombotic effect against one PH. The difference in antithrombotic effectiveness between PH + aspirin and the PH was 10 -11 per cent. So, we have established, that aspirin strengthens antithrombotic effect of low-molecular plant heparin.l

1432

SUCCESSFULL TREATMENT OF HEPARIN INDUCED THROMBOCYTOPENIA TYPE II AND THROMBOSIS WITH FONDAPARINUX IN A DIALYSIS PATIENT

E. Tekgunduz, S. Akpinar, E. Ozturk, G.E. Pamuk, B. Turgut, M. Demir

Trakya University Faculty of Medicine, EDIRNE, Turkey

Although rare heparin induced thrombocytopenia (HIT) type II is one of the most feared complications of heparin therapy. Unfractionated heparin is the standard anticoagulation used in hemodialysis sessions. Dialysis patients who are continually exposed to heparin are at risk for HIT. We report a 75-year-old male patient with acute-on-chronic renal failure who subsequently developed HIT while on hemodialysis. The patient admitted to our emergency department with dyspnea, tachypnea and bilateral low extremity edema. His physical examination and laboratory assessment revealed acute lung edema, uremic acidosis and acute-on-chronic renal failure requiring urgent renal replacement therapy. His history revealed hypertension for 15 years and diabetes for 1 year. A double lumen hemodialysis catheter was inserted into the left femoral vein and regular hemodialysis therapy with unfractionated heparin as anticoagulant was started. 10 days after starting hemodialysis the platelet count dropped from 504000/mm^2 to 47000/mm^2 and there were pain and swelling of his left leg. Doppler ultrasound examination showed left femoral vein thrombus formation. Thereafter enoxaparin therapy was started for deep vein thrombosis. Five days later when platelets were found to be 22000/mm^2 the patient was consulted with one of our hematology team members. As the patient had no other explanation for thrombocytopenia HIT type II was strongly suspected. Anti-heparin platelet factor-4 (Diagnostica Stago, France) complexes were positive (OD 2,375). Both functional assays, heparin-induced platelet aggregation test (HIPA) and C14-serotonin release assay were positive. Low molecular weight heparin therapy and unfractionated heparin during hemodialysis sessions were stopped. Fondaparinux 2.5 mg daily was started. We could not monitor the anti-Xa activity because of technical problems. During fondaparinux treatment platelet count increased to 193000/mm^2 and repeated Doppler ultrasound showed recanalization of left femoral vein thrombus. When the platelet count reached 100000/mm^2 oral anticoagulation with warfarin was initiated. The dose of warfarin was adjusted to maintain a target INR of 2.5. When INR was therapeutic for two consecutive days fondaparinux was stopped. During follow-up no new thromboembolic attack was observed. We present a patient with HIT type II and femoral vein thrombosis while on dialysis who was successfully treated with fondaparinux. In this case HIT type II and catheter-induced vessel wall damage were two independent risk factors for venous thrombosis. As HIT type II is a life threatening complication of heparin therapy all physicians using heparin anticoagulation should be aware of it. For all patients receiving unfractionated heparin alternate day platelet counts should be performed from days 4 to 14. As fondaparinux is too small to be recognized by the majority heparin-reactive antibodies it could be a reasonable alternative anticoagulant for symptomatic HIT type II patients where licensed drugs like lepirudin and danaparoid are not available.

1433

INCREASED RISK OF DEVELOPMENT OF HEPARIN INDUCED THROMBOCYTTOPENIA (HIT) IN ICU CRITICALLY ILL PATIENTS WITH VENOUS OR ARTERIAL LINES IN PLACE AND/OR NEED OF RENAL REPLACEMENT THERAPY WITH CONTINUOUS VENOUS-VENOUS HEMODIAFILTRATION (CVVHDF)

C. Mathas,S. Likousi, P. Paraskevopoulou, A.Vlahopoul, P.P. Paraskevopoulou

G.Hospital 'Agia Olga', ATHENS, Greece

To investigate whether the use of heparin as anticoagulant in either central venous,arterial or CVVHDF catheters ,as well as Low Molecular Weight Heparins(LMWH) increases the risk of HIT.Final objective is the follow-up and appropriate treatment of this critically ill population,given that early diagnosis of HIT reduces mortality from 30% to < 10%. Thirty (30) patients (17 men and 13 women)aged 31-87 years ,admitted in the ICU for various reasons during 2004-2005 were included in the study. At admission ,APACHE II score was <20 in 14 patients and > 20 in the remaining 16 patients.All patients had arterial or central venous catheters flushed with small quantities of unfractioned heparin.In 14 patients (group A) LMWH was administered in every day basis as prophylaxis from deep vein thrombosis(enoxaparine 2000-4000 IU/day), while 6 patients (groupB) underwent CVVHDF using unfractioned heparin as anticoagulant.Platelet measurements were performed in all patients at day 1st, 7th and 15th with hematology analyzer ADVIA120.Antibodies against the complex heparin-PF4 with elisa (Ass erachrom EPIA,Stago) were tested in all patients at day 9 and 16 after catheter insertion.

	Catheters +LMWH Group A n=24			*Catheters + CVVHDF Group B n=6*		
	1st day	7th day	15th day	1st day	7th day	15th day
Thrombopenia	-	1 (HIT)	1 (HIT)	-	-	-
HIT-IgG	-	10	+4	-	2	+ 1

From all patients studied (30), at day 7,12 patients from group A and 2 patients from group B were positive for HIT-IgG antibodies. In addition, at day 15 ,other 5 patients (4 from groupA and 1 from group B) were positive for HIT-IgG detection.Overall, 17 patients (56,6%) developed HIT-IgG antibodies (seroconversion).Thrombocyttopenia (HIT) was detected in 2 patients (6,6%). All patients with HIT-IgG antibodiew underwent vascular imaging (triplex) in order to exclude subclinical thrombosis.No correlation was found between severity score (APACHE II) and presence of HIT-IgG antibodies. According the results of this study ,combination of heparinized catheters or use of LMWH seems to increase the incidence of HIT(6,6%) as well as the development of HIT-IgG antibodiew (seroconversion) in about 58,3% of patients. On the other hand ,combined use of heparinised catheters and CVVHDF filters ,seems to increase the presence of HIT-IgG antibodies (seroconversion) in high percentage of patients (50%). The management of ICU patients with HIT includes :

- Discontinuation of LMWH
- Flushing catheters with normal saline
- Use of filters without need of heparin.

1434

SEMINAL FACTOR VII AND VIIA: THE ULTIMATE EVIDENCE ON THE PRESENCE OF THE TISSUE FACTOR DEPENDENT PATHWAY IN HUMAN SEMEN

A. Lwaleed,[1] B. Lwaleed,[1] G. Delves,[2] A. Goyal,[2] R. Greenfield,[3] A. Cooper[2]

[1]*Southampton University Hospital NHSTrust, SOUTHAMPTON, United Kingdom;* [2]*University of Portsmouth, PORTSMOUTH, United Kingdom;* [3]*American Diagnostica Inc, STAMFORD, CT, USA*

Backgrounds. Human semen spontaneously coagulates into a semisolid mass and then wholly liquefies in a process that may have some similarity to that of blood. Besides other active components of the haemostatic system, semen contains a significant amount of functional tissue factor (TF). Aim: To investigate the presence of Factor (F) VII and FVIIa in human semen. *Materials and Methods.* Using a PT/APTT one stage factor assay and an Imubind™ FVIIa ELISA-assay, FVII and FVIIa levels were assessed in 97 semen specimens obtained from sub-fertile, normally fertile, fertile sperm donors and vasectomized subjects. Results. FVII and FVIIa were quantifiable in human semen. The mean FVII levels were 4.4 IU/dl and FVIIa were12 ng/ml. Despite the observed variations in seminal FVIIa levels we found no significant differences in FVIIa levels among the studied groups. Seminal FVIIa levels showed a significant positive association with semen liquefaction time, sperm motility and semen volume. The anti-sperm antibodies and sperm-agglutination groups also showed raised FVIIa levels. We found no relationship between FVIIa levels and total sperm concentration (density), sperm counts per ml, sperm progression and days of abstention. *Conclusion.* The present finding reinforces the concept of an active clotting system in human semen, not least the presence of the TF-dependent pathway.

1435

NO EFFECT B-VITAMIN SUPPLEMENTATION ON MARKERS OF INFLAMMATION IN PATIENTS WITH VENOUS THROMBOEMBOLISM

R.C. da Silveira,[1] C.A. Rodrigues,[1] M.A.E. Noguti,[1] V.M. Morelli[1], V. D'Almeida,[1] A.A. Garcia,[2] F.H.A. Maffei,[3] D.M. Lourenco[1]

[1]*Universidade Federal de Sao Paulo, SAO PAULO, Brazil;* [2]*Universidade de Sao Paulo, RIBEIRAO PRETO, Brazil;* [3]*Universidade Estadual Paulista, BOTUCATU, Brazil*

Backgrounds. Mild hyperhomocysteinemia is associated with an increased risk of venous thromboembolism (VTE) and other cardiovascular diseases. Recent studies have suggested a role of inflammatory markers in the etiology of VTE and elevated homocysteine levels may contribute to low-grade inflammation. Vitamin supplementation with folic acid and B-vitamins was previously shown to decrease homocysteine levels. *Aims.* This study was designed in order to evaluate the correlation between homocysteine and markers of inflammation and to evaluate the effect of vitamin supplementation on these markers in patients with VTE. *Methods.* This study was a multicentre, randomized, double-blind, placebo-controlled trial. We randomized 105 patients with a first event of objectively confirmed VTE, aged between 18 and 70 years, to receive either vitamin supplementation (folic acid 5 mg, vitamin B6 50 mg and vitamin B12 0.4 mg) or placebo. Blood was collected at randomization and 8 weeks after the intervention period. *Results.* Ninety-nine (94.3%) completed the 8-week period of treatment. There was no difference in the levels of C-reactive protein (hsCRP) between patients with homocysteine above the highest tertile (12.6 μmol/L) and patients with below the lowest tertile (9.9 micromol/L). Median hsCRP was 0.28 mg/dL in patients with higher homocysteine levels and 0.19 mg/dL in patients with lower homocysteine levels (p=0.29). There was also no difference in the levels of interleukin-8 (52.5 and 59.5 pg/mL, respectively, p=0.51) between the two groups. In the patients treated with vitamins, there was a 29% decrease in the homocysteine levels. However, the levels of hsCRP and IL-8 did not change both in the vitamin and in the placebo-treated patients. Besides, treatment with vitamins had no effect on these markers even in patients above the highest tertile of homocysteine. *Conclusions.* In patients with VTE, higher homocysteine levels are not associated with increased levels of inflammation markers and homocysteine reduction by B-vitamin supplementation had no effect on these markers.

1436

PRIMARY HEMOPHAGOCYTIC SYNDROME CASE REPORT

N. Sarper, E. Zengin, F. Corapcioglu

Kocaeli University, KOCAELI, Turkey

An 11 year-old white girl with good features presented with a history of fever, somnolans, jaundince and petechia. She had a past history of jaudince and elevation of transaminases five months ago which resolved without therapy. There was hepatomegaly and splenomegaly 3 and 4 cm respectively. Laboratory findings were: Hb:8.4 g/dl, WBC: 1600/mm3, PLT:1800/mm3, ALT:290 U/L, AST:129 U/L, T.bilirubin:12.5 mg/dl, D.bil:4.5 mg/dl, PT:17.4 sec, PTT:42 sec, INR:1.5, fibrinogen: 90 mg/dl, serum ammonia: 150 mg/dl. Cerebrospinal fluid examination was normal. Clinical presentation was suggesting hemophgocytic lymphohistiocytosis (HLH) but bone marrow aspiration did not confirm the diagnosis. Marrow biopsy revealed hemophagocytosis. Hepatit B, Hepatit A, CMV, EBV, HSV, parvovirus serology did not show acute infection. Hepatit A IgG was positive, HCV-RNA was negative. HLH-94 protocol was started and fever, hepatosplenomegaly and somnolans subsided but only partial hematological remission could be achived (Hb:6.1 g/dl, WBC:4900/mm3, PLT:47000/mm3). ATG 10 mg/kg/day for three consecutive days were also administered and complete hematological and clinical remission was achieved. Parents were cousins but there was no history of similar disease. Genetic study could not be performed to confirm primary HLH. Following three uneventful years the patient was refered to our center again with a 5 month history of bilateral abduscens paralysis and ataxia. Cranial MRI showed increased T2 signal in cerebellar, supratentorial areas, right occipital deep white matter, bilateral talamus and centrum semiovale and diffuse cerebellar and mild cortical atrophy. HLH-94 protocol was again started but progression was seen with head tremor, generalized clonic convulsion, fever (39C axillar) and cytopenias. Hb:10.6 g/dl, WBC:2130/mm^3, ANC:678/mm^3, PLT:78400/mm^3, ALT:1586 U/L, AST:802 U/L, Fibrinogen 440 mg/dl, ferritin:160 ng/mL Genetic study showed homozygot perforin mutation that leads to aminoacid exchange from (Val50Thr). We are planning allogeneic stem cell transplantation from siblings if they do not show the same homozygot perforin mutation because clinical presentation might be late as seen in the patient. HLH must be remembered in the differential diagnosis of, fever, cytopenias, splenomegaly, hepatic failure and/or neurological symptoms. Delay in diagnosis may impair outcome. Allogeneic stem cell transplantation is the only curative therapy in primary disease.

1437

HERPES GROUP VIRUS INFECTIONS AND GRANULOCYTOTOXIC ANTIBODIES IN CHILDREN WITH NEUTROPENIAS

E.A. Mamedova, M.N. Vasilyeva, T.V. Polovtseva, N.A. Finogenova

Federal Clinical Research Center of Pedi, MOSCOW, Russian Federation

Relation between the duration of granulocytotoxic antibodies (GCTA) circulation and the presence of herpetic infection has been studied in children with immune neutropenias. Group 1 consisted of 15 herpes group virus-infected children with immune neutropenia aged 4 to 24 months; virus infections included cytomegalovirus (n = 10), Epstein-Barr virus (n = 1), herpes simplex virus 1 or 2 (n = 2) and mixed infection (n = 2). GCTA circulation lasted for 0.5 to 14 months (6.96 ± 0.33). GCTA titers ranged from 1:2 to 1:64. No correlation between GCTA titers and duration of GCTA circulation has been revealed. Group 2 consisted of 18 children aged 6 to 12 months with immune neutropenia and no markers of herpes group viruses. GCTA circulation lasted for 0.5 to 5 months (1.67 ± 1.25). GCTA titers ranged from 1:4 to 1:156 and, similarly to those in Group 1, caused no effect on the duration of GCTA circulation. Thus, statistically significant difference in duration of GCTA circulation ($p < 0.001$) between the studied groups has been found; this result indicates the presence of a pathogenetic role of herpes group viruses in the immune conflict in children with immune neutropenias.

1438

THE VALUE AND LIMITATIONS OF WBC DIFFERENTIAL FLAGS PROVIDED BY THE AUTOMATED HEMATOLOGY ANALYSER SYSMEX XT 2000I

D. Vasilache, D. Ostroveanu, D. Bratu, V. Moraru

Fundeni Clinical Institute, BUCHAREST, Romania

Backgrounds. SYSMEX XT 2000i is a fully automated hematology

analyser with a throughput of 80 samples per hour. The analyser can provide up to 30 parameters including CBC and complete 5-part leucocyte differential using flow cytometry by semiconductor laser enabling a sophisticated analyses based on RNA/DNA content, cell size and inner cell complexity. The analyser detects the presence of abnormal or immature WBC providing a list of suspect messages generated from abnormal cell locations on WBC/DIFF scattergram. *Aims.* The present study evaluates the diagnostic performance of Sysmex XT 2000i in detection of abnormal or immature WBC in comparison with manual microscopy review and also the value of flags in leucocyte count. *Methods.* In this study were included 100 samples. All venous blood specimens were colected from 100 patients admitted in Hematology Department between July 2005-February 2006 and diagnosed as acute leukemia (44 cases), CLL(15), malignant lymphoma(11), chronic myeloproliferative disease(9), MDS(8), anemia(5), trombocytopenia(3), infectious mononucleosis(2), HCL(1), ITP(1), MM(1).Clinical sensitivity and specificity of suspect flags of XT were assesed by comparison with microscopy differential counts. *Results.* Sysmex XT 2000i generated messages of blasts in 95% of cases confirmed in 61% with optic microscopy. In the group of 44 cases with acute leukemia, XT flagged blasts in 41 cases (93%) comparative with optic microscopy which detects blasts in 43 cases (98%). In MDS cases (8), 5 samples(62%) were flagged on XT and optic microscopy confirmed presence of blasts in all 8 cases. 15 samples of CLL were false positive for blasts on XT, in all cases we found mature lymphocytes. *Conclusions.* Samples flagged with *blasts* on XT need a manual microscopy review.Sysmex XT 2000i shows high sensitivity (95%) and lower specificity (50%) in detections of blasts, provide reliable results and a WBC differential comparable with optic microscopy.

1439

ETHICS AND QUALITY OF THE WEBSITES THAT OFFER INFORMATION ABOUT LYMPHOID NEOPLASMS IN SPANISH LANGUAGE

A. Garcia-Nieto, F.J. Capote

Hospital Puerta Del Mar, CADIZ, Spain

The use of emerging information and communication technology, especially the Internet, is changing how people give and receive health information and health care. The Internet offers real potential to improve well-being. All who use the Internet for health-related purposes must join together to create an environment of trusted relationships to assure high quality information and services; protect privacy; and enhance the value of the Internet for both consumers and providers of health information, products, and services. Although recent studies have begun to document Internet use among cancer patients, few published studies have specifically examined the ethics and quality of websites. To identify the websites that contains information in Spanish language about lymphoid neoplasms and to evaluate the compliance with ethical and quality standards. Identification of the websites: the spanish term *linfoma* was written in the box of search of Google, Yahoo, Altavista, Excites, Lycos, Hotbot and Wanadoo. Analysis of ethical and quality standards of European commission (e-Europe 2002), Internet Health Care Coalition (eHealth Code of Ethics) and certificates of internet quality agencies and Medical Associations: HONcode (Health On the Net Foundation), WMA (Web Médica Acreditada), pWMC (proyecto Webs Médicas de Calidad) and IQUA (Internet Quality Agency). Twenty-one websites has been identified: scientific societies (6), personal web pages (5), foundations (4), pharmaceutical industry (3), patients association (2) and others (1). These internet sites provide information on lymphoid neoplasms (10) or cancer (11). The results and the site's ranking have been different according to the search engine used. Compliance with all criteria of e-Europe 2002: Transparency and honesty 42% of the analyzed pages; Authority 38%; Privacy and data protection 62%; Updating of information 28%; Accountability 5% and Accessibility 23%. Compliance with all criteria of eHealth Code of Ethics: Candor 62% of the analyzed pages; Honesty 66%; Quality 14%; Informed Consent 38%; Privacy 33%; Professionalism in Online Health Care 42%; Responsible Partnering 66% and Accountability 47%. Only eleven websites have Certificates of internet quality agencies and/or Medical Associations: 7 (HONcode), 1 (WMA), 1 (HONcode, WMA and pWMC) and 2 (HONcode, WMA, pWMC and IQUA). There are few websites that provide information about lymphoid neoplasms in Spanish language and they have important deficiencies in the evaluated aspects. On search engines the site's ranking does not keep relation with the quality from these pages. A part of the information is not generated by the Spanish sites and it is obtained by links to Spanish texts of pages in English language; this behaviour impede the users to examine the compliance with ethical and quality standards of these pages We consider, in order to improve and guarantee the ethics and quality of the information to patients and caregivers, that is very important the development of agencies for the evaluation and certification.

1440

ORAL VORICONAZOLE AS SECONDARY PROPHYLAXIS DURING ALLOGENEIC STEM CELL TRANSPLANTATION IN A PATIENT WITH PREVIOUS SEVERE INVASIVE FUSARIUM SP INFECTION

L.C. Palma, M.I.A. Madeira, M.C. Favarin, C.E.E. Velano, L.F. Dalmazzo, F. Pieroni, G. Navarro, A.B.P.L. Stracieri, D. Oliveira, M.C. Moraes, L.L. Figueiredo, M.C.T. Pintao, L.C.O. Oliveira, J.C. Voltarelli, B.P. Simoes

University of So Paulo, RIBEIRAO PRETO, Brazil

Invasive fungal infections (IFI) represent one of the most challenging complications in patients submitted to stem cell transplantation (SCT). Invasive fusariosis have a high mortality rate in immunocompromised patients. Retrospective analysis of 69 brazilian patients (Nucci et al, CID 2004) showed a median survival of only 13 days. None of this patients, although received a newer antifungal drug, such as Voriconazole. Some studies have showed the safety and efficacy of Voriconazole as a secondary prophylaxis during SCT in patients with previous Aspergillus sp infection. However, to our knowledge, the use of Voriconazole as a secondary prophylaxis in SCT for patients with previous invasive Fusarium sp infection has not been reported yet. *Case Report.* A 49 years old man, who received a SCT in 2001, developed 4,5 years thereafter a secondary AML in the cells of the donor (*Pieroni et al. BMT in press*). An attempt to induce a remission with standard treatment (Cytarabin and Daunorrubicin) failed. A second course was started. Routine chest evaluation during febrile neutropenia showed halo sign on computed tomography (TC) and deoxicolated Amphotericin was started. After two days, with the appearance of skin lesions and important myalgia a clinical suspicion of fusariosis was made and oral Voriconazol was started. Fusarium sp blood culture sample confirmed the diagnosis of invasive fusariosis. He achieved complete remission after salvage chemotherapy and used oral Voriconazole for 2 months. The latest pulmonary CT scans showed a very small residual lesion in the lower lobe of the right lung. With two other sibling identical donors, we submitted him to a second SCT with a different donor. During the whole conditioning period (Bussulfan 16 mg/kg and Fludarabine 120 mg/m^2) and until day + 6 he received 400 mg of oral Voriconazole. Liver and renal test were undertaken daily, and Cyclosporine level were measured twice a week. With a slight increase in bilirrubin on day + 6 (6,48 mg/dL) Voriconazole was stopped for two days and reintroduced two days later half the dose (200 mg). He had no further complications, except for a grade II mucosites. Bone marrow take was on day +18. Routine weekly CT chest scans don't reveal any radiological signal of Fusarium sp infection reactivation. *Conclusions.* At the best of our knowledge this is the first described case of successful secondary prophylaxis with Voriconazole in a patient with previous severe disseminated fusariosis submitted to a stem cell transplantation. Since fusarium infections have a trimodal distribution after SCT (Nucci et al. CID 2004) further cautious follow up will be necessary in this case. But we can conclude that oral Voriconazole seems to be an important new drug that can be safely used for secondary prophylaxis during SCT in patients with previous invasive Fusarium sp infections.

1441

EFFICACY OF PAROMOMYCIN AND / OR AZYTHROMYCIN IN HEMATOLOGICAL PATIENTS WITH CRYPTOSPORIDIASIS

C. Ulibarrena, J.L. Sastre, M.S. Garcia-Torremocha, V.G. Aneiros, C. Mouronte

Complexo Hospitalario de Ourense, OURENSE, Spain

Cryptosporidum parvum is a widespread parasite of the Apicomplexa genre, with a fecal-oral way of transmission and capable of overcoming chlorination of water. Intestinal cryptosporidiosis as a cause of severe diarrhea is not uncommon in underveloped countries. Since the 80's, a rising incidence occurred in Western countries, related to HIV pandemic. There are only isolated cases of the infection in the setting of hematological disease. Diagnosis requires a high level of suspicion and several explorations, often unsuccessful. There is no consensus on therapy, although all options seem rather unsatisfactory: nitazoxanide or long-term use of Paromomycin, Espiramycin and/or Azythromycin p.o. have been proposed, without certainty of eradication. *Case 1*: 66 yr.-old male. Diagnosed of myelodysplastic syndrome, AREB•5q-, with multiple

infectious episodes. In March-02, he was admitted to Hospital with fever, loss of weight (12 kg), diarrhea with >10 depositions/day, of liquid orange stools devoid of blood, tenesmus and abdominal tenderness with peritoneal signs. X-ray of abdomen showed diffuse dilation of gut. Abdominal ultrasound scan and TAC revealed scarce free liquid and thickening of colonic wall. Colonoscopy: replacement of normal mucosa by multiple nodules, resembling sessile polips; a biopsy of one of them was informed of 'minimal inflammatory changes'. Conventional microbiologic studies rendered no result (cultures, C. difficile toxin, serologies, search for virus and parasites). Specific search for C. parvum (modified Ziehl's stain) was positive in 3 samples. The CD4+ lymphocyte count was 400/μL. Therapy: paromomycin, 1 g p.o. b.i.d. and diet supplementation with *Lacto-bacillus sp*. Resolution of diarrhea, a significant weight gain and improvement in performance status was attained in the following weeks. *Case 2*: 18 yr.-old male. Diagnosed of Hodgkin's disease, NS-subtype, stage IV-B, refractory to several lines of chemotherapy, including BEACOPP, ESHAP/MINE and a gemcitabine 'based scheme. In April-05, he was admitted to Hospital because of protracted fever, diarrhea with green liquid stools, loss of 4 kg in a single week, diffuse abdominal pain, vomiting and tenesmus. Conventional microbiologic studies were also inconclusive. Considering the former case, we asked again for a C. parvum search in stools, which was clearly positive. Therapy with azythromycin (5 days) and paromomycin (14 days) was undertaken, after which diarrhea and fever, as well as the other symptoms disappeared in this period; complete clearance of parasite cysts in stools could be demonstrated. A significant recovery of nutritional status was also accomplished. 1.- Conventional methods for detecting parasites in stools may not detect C. parvum. This protozoan must be suspected when no diagnosis can be drawn after a complete set of explorations, and an intentional search with specific stains. Although nitazoxanide has been approved for Cryptosporidiasis, this drug is not available in Spain yet. We believe that this simple combination, i.e.. azythromycin, paromomycin and diet supplementation is a suitable option for an, otherwise emaciating, unusual form of infectious diarrhea in hematological patients.

1442

ATYPICAL EOSINOPHIL DISTRIBUTION OBSERVED IN PATIENTS WITH MALARIAL INFECTION WHEN USING SYSMEX XE-2100

J. Cha,[1] G.B.R. Park,[1] C.J. Park[2]

[1]*Chung-Ang University, SEOUL, South-Korea;* [2]*Ulsan University Seoul Asan Hospital, SEOUL, South-Korea*

Backgrounds. The incidence of malaria has been increasing in civilian population and the prevalent area being more wider in Korea. Malaria must be recognized promptly in order to treat the patient in time and to prevent further spread of infection in the community. *Aim*. Malaria can be suspected based on the patient's symptoms and the physical findings at examination. However, for a definitive diagnosis to be made, laboratory tests must demonstrate the malaria parasites or their components, which are time consuming and need expertise. As it is likely that general screening tests like a complete blood cell count are always undertaken for patients who present with pyrexia, it can be expected that attention to any abnormalities found in automated hematology analyzer can decrease a delay in the diagnosis of malaria if such a diagnosis was not initially considered.

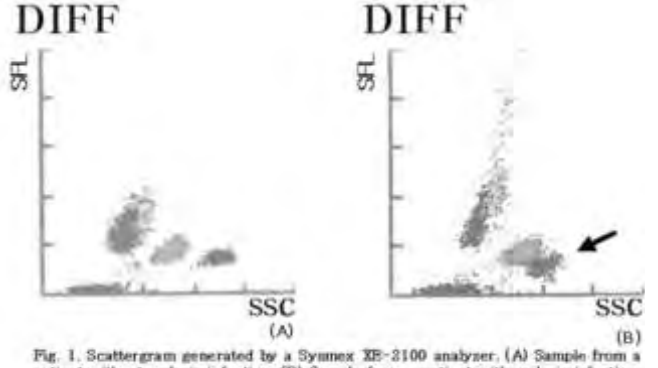


Fig. 1. Scattergram generated by a Sysmex XE-2100 analyzer. (A) Sample from a patient without malaria infection. (B) Sample from a patient with malaria infection, showing atypical distribution of eosinophils (arrow). (clusters: skyblue: neutrophils, red: eosinophils, green: monocytes, pink: lymphocytes)

Methods. Hematological analysis using Sysmex XE-2100 (TOA medical Electronics, Kobe, Japan) and Advia 120 (Bayer Diagnostics, Tarrytown, NY, USA) was performed on samples positive for malarial parasite. *Results.* We found 3 peculiar patients with P. vivax malaria who had pseudoeosinophilia determined only when using Sysmex XE-2100. Although eosinophilia of 5.4%-24.3% was found in 3 patients when measured by Sysmex XE-2100, eosinophilia was not found either when measured by Advia 120 or read by microscopy. As a result of reviewing the scattergram generated by Sysmex XE-2100, atypical eosinophil distribution was placed more closely to the neutrophil distribution than typical eosinophil distribution in the WBCs scattergram (Fig. 1). This atypical eosinophil distribution was due to the presence of hemozoin-containing neutrophils. It was concluded Sysmex XE-2100 analyzer showed erroneously high eosinophil counts. *Summary/Conclusions.* It is feasible that reading the WBCs scattergram to find a certain hematologic abnormality such as atypical eosinophil distribution as a result of hemozoin-containing neutrophils may contribute to the diagnosis of malaria especially for patients unsuspected.

1443

USEFULNESS OF THE VORICONAZOLE PLASMA LEVELS MONITORING IN HEMATOONCOLOGICAL PTS TREATED BY ORAL FORM OF THE VORICONAZOLE

Z. Racil, L. Malaskova, B. Wagnerova, I. Kocmanova, M. Doubek, J. Mayer

University Hospital Brno, BRNO, Czech Republic

Backgrounds. Voriconazole, a new azole antifungal agent, is widely used in hematooncological pts. Even the bioavailability of the voriconazole oral form reaches 90%, several situations can lead to the impairement of the drug absorption. Even a lot of discussions about usefulness of the voriconazole plasma levels monitoring, we decided to monitor levels of this drug in our pts to confirm adequate absorption in these severely ill pts. *Methods.* In all pts treated with oral voriconazole from 8/2005 to 2/2006 steady-state trough plasma voriconazole levels were measured using an HPLC assay. *Results.* 49 samples from 22 pts were tested. Pts had drug levels checked once (n=8), twice (n=7) or =/> 3 times (n=7) 4-86 days (median 11) after starting voriconazole or dose modification. Mean and median plasma levels were 1,1 and 0,75 microgram/ml (range: < 0,2 - 5,41 μg/mL). 19 samples (39%) from 10 pts (45%) were < 0,5 microgram/ml (possibly below the *in vitro* MIC90 for Aspergillus sp.) and 12 samples (24%) from 7 pts (32%) were < 0,25 μg/mL (possibly below the mean in vitro MIC90 for Candida sp. in our dept.). Potentially impaired absorption of the voriconazole (due to worsened intestinal peristalsis or aplication through NG tube) or using of reduce dose of voriconazole as a reason for lower drug steady-state plasma levels were indentified in 7 of 12 samples (58%) with voriconazole plasma level < 0,25 and in other 2 of 7 samples (29%) with voriconazole plasma level between 0,25 - 0,5. Interestingly, in 5 of 12 samples (42%) with voriconazole plasma level < 0,25 and in other 5 of 7 samples (71%) with voriconazole plasma level between 0,25 - 0,5 the reason for the insufficient drug level in plasma were not identified. Hepatic CYP2C19 genetic polymorphism with differences in drug metabolisation can be the possible explanation. The dose of oral voriconazole was increased in 3 pts, that leads to drug steady-state plasma level increase with mean 2,47 microgram/ml. *Conclusions.* Voriconazole plasma levels after the use of oral form of the drug in hematooncological pts vary significantly and plasma levels monitoring can help to clinicians to confirm achievement of the therapeutic levels of voriconazole especially in pts with gastrointestinal impairment. This approach needs farther evaluation.

Funding: Supported by NR8452-3/2005

1444

JC PAPOVAVIRUS LEUCOENCEPHALOPATHY AFTER TREATMENT WITH CHEMOTHERAPY AND RITUXIMAB

J. Rey, N. Bouayed, D. Coso, J.A. Gastaut, R. Bouabdallah

Institut Paoli-Calmettes, MARSEILLE, France

Background. Progressive multifocal leucoencephalopathy (PML) is a rare demyelinating infection of the central nervous system caused by the JC papovavirus usually seen among immunocompromised patients. The most common underlying immunosuppressive illness is AIDS. However, PML may be seen among patients with lymphoproliferative disorders and immunosuppression induced by chemotherapy. Recently, an association between PML and rituximab with autologous or allogeneic transplantation has been discussed. *Aims*. We report the case of a woman with a mantle cell lymphoma who developed PML after a combination of chemotherapy with rituximab. *Methods*. A 67 year old woman was

diagnosed with mantle cell lymphoma because of splenomegaly and hyperlymphocytosis. Staging shows a stage IV with bone marrow involvement. The patient was treated with a combination of rituximab (375 mg/m^2 D1) and chemotherapy with standard CHOP: cyclophosphamide 750 mg/m^2 D1, doxorubicin 50 mg/m^2 D1, vincristin 2 mg D1, and oral prednison 100 mg D1 to D5 given in 3-week cycles. She received eight cycles of treatment. Evaluation after the eight cycles shows a situation of complete remission. One month after the last chemotherapy, the patient presents rapidly psychiatric disturbances with speech dysfunction and paranoia delirium. PML was suspected after magnetic resonance imaging with frontal and temporal leucoencephalopathy. JC viral DNA was detected in the cerebrospinal fluid. HIV serology was negative. Low level of CD4, CD8, B lymphocytes and NK cells was noted. A treatment with cidofovir was started. Two months after the beginning of symptoms, neurological disturbances were stable. *Results*. A few cases of PML were recently described in patients who were treated with chemotherapy, transplantation and peritransplantation rituximab. A direct association between rituximab and PML remains speculative. Moreover, the patients reported were often in relapse, heavily pre treated. Our patient is, to our knowledge, the first case of PML after a combination of CHOP with rituximab, in first induction procedure. *Conclusions*. Unusual viral infections were recently described in patients treated with high dose chemotherapy and rituximab. Although the contributory role of rituximab remains speculative, our additional case highlights the need for an accurate surveillance, even in patients not heavily pre treated, in first induction with CHOP and rituximab.

1445

VARIABLE RESPONSE TO CURRENT TREATMENT OPTIONS IN HYPEREOSINOPHILIC SYNDROME (HES)-A SINGLE CENTRE EXPERIENCE

S. Mitra, R. O'Donnell, P.T. Murphy

Beaumont Hospital, DUBLIN, Ireland

Backgrounds. Hypereosinophilic syndromes (HES) refers to a heterogenous group of disorders characterised by marked blood eosinophilia (>1500/cu mm) and tissue eosinophilia (lasting for more than 6 months),in the absence of other eitiologies for eosinophilia, resulting in end organ damage. HES may be a reactive condition or a chronic myeloproliferative disorder(with evidence of clonal proliferation). Reactive eosinophilias are due to release of cytokines (IL-3 IL-5,GM-CSF etc) and the common causes are parasitic (helminthic) infections,allergic diseases,vasculitides drug reactions and malignancies. Clonal eosinophilias are those in which the eosinophilia is a part of a clonal haematological malignancy,which is very often associated with the fusion gene FIP1L1-PDGFRα which causes the generation of a constitutively active Tyrosine Kinase. Several visceral complications like cardiomyopathies, nervous system involvement (e.g. paraparesis,cerebral infarction,eosinophilic meningitis etc) are often fatal illnesses. Treatment modalities for HES includes corticosteroids, chemotherapeutic agents (hydroxyurea, cyclophosphamide vincristine) and α-interferon. Newer treatment modalities including tyrosine kinase inhibitors(eg Imatinib mesylate) and monoclonal anti-IL5 antibodies are now available. Patients carrying this fusion gene respond well to the Tyrosine Kinase Inhibitor Imatinib.Some patients with HES ,that are negative for this fusion gene may also respond to Imatinib,suggesting that in such cases other Tyrosine Kinases may be dysregulated. *Aim*. Retrospective review of the variable response of 5 patients with HES (over a period of 6 months), to current treatment modalities. *Methods* .The 5 patients (4 Male, Female; age range 37-80 yrs; mean age 56 yr) presented with eosinophilia in the range 2600-12,000/cumm. A response to treatment was defined as Eosinophil count< 1500 /cumm or Eosinophil count < 5% of the total leucocyte count in the peripheral blood. 3 of the 5 patients received Imatinib as initial treatment. 1 patient initially had Methyl Prednisolone followed by Imatinib and 1 patient (aged 80 yr) was treated with Hydroxyurea initially. Results. Two of the four patients receiving Imatinib responded to it.Of the 2 patients not responding to Imatinib,1 responded partially to Hydroxyurea and the other did not respond to monotherapy with steroid or α-interferon. However the latter eventually responded to a combination of steroids and α-interferon.The patient who had initial treatment with hydroxyurea responded well. Of the 5 patients 1 was equivocal (possibly false positive) for the FIP1L1-PDGFRa fusion gene. Two were negative and two were not tested.Of the 2 that were negative 1 responded to Imatinib (see Table). *Summary*, Thus response of HES patients to the various treatment modalities is variable and often unpredictable.A trial of Imatinib is worth considering in all cases. In case that are refractory to monotherapy with Imatinib, steroids and α-interferon, a combination of the last two agents may be tried. In difficult resistant cases of HES, monoclonal anti IL 5 may be tried.

Table 1. Hypereosinophilic syndrome.

Patients	*Age (years) Sex Male/Female*	*Clinical Presentation*	*Eosinophil Count@ presentation (/cu/mm)*	*FIP1L1 PDGFRa Status*	*Initial Treatment*	*Response To Imatinib*	*Second line Agents*
1	37/F	History	2600	Equivocal	Imatinib	No	Prednisolone
2	43/M	CVA - Right Lacunar Infarct Mitral valve Endocarditis	12.000	Negative	Methyl Prednisolone (followed by Imatinib)	Yes	Not applicable
3	59/M	-	3500	Not tested	Imatinib	Yes	Not applicable
4	63/M	Atrial Fibrillation	3000	Negative	Imatinib	No	Hydroxyurea
5	80/M	Alzheimer's Disease	3500	Not tested (tryptase level High)	Hydroxyurea	Not applicable	Not applicable

M: Male; F: Female.

1446

SIBILING MATCHED HSCT CAN RESTORE HEMATOPOIESIS AND STROMAL TISSUE IN PATIENTS WITH IDIOPATHIC MYELOFIBROSIS

H.M. Sedzimirska, A.L. Lange

Lower Silesian Center for Cellular Trans, WROCLAW, Poland

Natural history of idiopathic myelofibrosis (IFM) is hardly modified by conventional therapy. Neither hydroxycarbamid nor IFNa and busulfan can restore normal hematopoietic function. Fibrosis of the marrow constitutes a characteristic feature of the disease.The parenchymal tissue lesions could make decision of hematopoietic stem cell transplantation (HSCT) more difficult, as it is not know, whether normal function of marrow suported tissue could be restored after transplantation in advanced cases. Since 2000 year five patients with IFM received sibiling matched HSCT (F/M 4/1, age 29 - 55 yrs, median age 46, all patients were in 3 stage of the disease according to the WHO pathological staiging). All patients fulfiled 4 diagnostic criteria established by PVSG and were in high or intermediate risk group of the disease according to the Dupriez prognostic scoring system. Two of them received myeloablative conditioning BuCy2, three nonmyeloablative conditioning: Bu or Mel , Flu and ATG prior to PBPC transplantation. One patient died 30 days post transplantation in the course of EBV reactivation (7345 viral copies/ 100.000 cells) with allergic vasculitis et hemolysis. Four patients are alive and well from 3 to 70 months post HSCT. Hematological recovery was prompt and followed by resolving of fibrosis easily seen as soon as 30 days post transplant. Normal marrow trephine biopsy pictures were found by six month post transplant. However, proportion of mesenchymal stem cells (CD45-, CD34-, CD105+, CD 73+, CD 90+) was lower in the marrow of IMF patients as compared to CML cases at the same time post transplant. All patients including fatal one were full chimera by one month post HSCT. In conclusion; normal hematopoietic and stromal tissue can be restored by HSCT in IMF cases at advanced stage.

1447

DETECTION OF JAK2V617F MUTATION IN MYELOPROLIFERATIVE DISORDERS: CLINICAL CORRELATIONS.

L. Bizzoni,[1] A. Tafuri,[1] A. Fama,[1] S. Trasarti,[1] E. Calabrese,[1] A. Levi,[1] R. Crispino,[1] C. Santoro,[1] A. Rago,[1] M. Breccia,[1] R. Latagliata,[1] G. Ruscio,[2] A. Zeuner,[2] M.G. Mazzucconi,[1] R. Foà,[1] G. Alimena[1]

[1]Hematology, ROME, Italy; [2]Istituto Superiore di Sanità, ROME, Italy

Ph-negative myeloproliferative disorders (MPD) include three major diseases represented by polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IM). All reflect clonal transformation of a multipotent hemopoietic progenitor. Overlapping clinical features and undefined pathogenesis are responsible for diagnostic and prognostic problems. Recently, several groups have identified a frequent single point mutation (V617F) in the JAK2 gene (*JAK2V617F*), sug-

gesting a common pathogenesis which involves the cytoplasmic tyrosine kinase JAK2 in these disorders. To evaluate the frequency of JAK2V617F status and possible correlation with clinical features in PV, TE and IM. We studied 59 patients, 29 males and 30 females, median age 52.8 years (range 22-83), diagnosed with MPD. According to PVSG criteria, patients were defined as PV (25), ET (27) and IM (7). At the time of the study, patients median follow-up was 52 months (range 3.5-307). Genomic DNA was extracted by standard procedures from peripheral blood granulocytes. The presence of the JAK2V617F was determined with the JAK2 activating mutation assay (InVivoScribe Technologies, San Diego, CA) based on BsaXI digestion of a PCR product encompassing the site of mutation. Among PV patients, the JAK2V617F mutation was detected in 20/25 cases (80%), with 14 of them (70%) being heterozygous and 6 (30%) homozygous. Comparison between JAK2V617F patients (group A) and JAK2 wild-type patients (group B) did not reveal significant associations with age, gender, hemoglobin levels and platelet count at the time of diagnosis. In contrast, a trend-association was found between median leukocyte count (group A: 9.8 x 103/µl vs. group B: 7.4) (p=0.07) and hematocrit (group A: 57.0% vs. group B: 52.9) (p=0.08). Spleen enlargement was only observed among mutated patients (8/20, 40%). Also thrombotic events were only registered in the JAK2 V617F patients (3/20, 15%). Among patients diagnosed with ET, the JAK2V617F mutation was detected in 20/27 cases (74%) and all were heterozygous. Comparison between ET groups A and B did not reveal significant associations with age, gender, leukocyte count. Instead, a trend-association was found for median hematocrit (group A: 40.7% vs. group B: 38.1) (p=0.07), median hemoglobin levels (group A: 14gr/dl vs. group B: 13.1) (p=0.06), median platelet count (group A: 878 x 103/µl vs. group B: 1.449) (p=0.09). Moreover, thrombotic events were observed only in group A patients 2/20 (10%). Among patients with IM, the JAK2V617F mutation was detected in 2/7 cases (28.6%), one heterozygous and one homozygous. The IM JAK2 V617F mutation homozygous patient had in the history a thrombotic event. In our series the JAK2V617F mutation was a very frequent clonal abnormality in PV (80%) and ET (74%) patients, while it was detectable in a smaller proportion of IM patients (28%). The homozygous JAK2V617F status was only found in PV (6/20) and in IM (1/2), while it was never detected in ET patients.

1448

EVALUATION OF THE BRITISH SOCIETY FOR HAEMATOLOGY CRITERIA FOR THE DIAGNOSIS OF POLYCYTHEMIA VERA

M.J. Percy,[1] E.A. Arnold,[1] R.C. Turkington,[1]
R.J.G. Cuthbert,[1] L.A. Ranaghan,[1] M.F. McMullin[2]

[1]*Belfast City Hosptial, BELFAST, United Kingdom;* [2]*Queen's University, BELFAST, United Kingdom*

Backgrounds. Polycythaemia vera (PV) is a myeloproliferative disorder in which the dominant feature is excessive erythropoiesis resulting in a raised red cell mass. Criteria have been established by the Polycythaemia Vera Study Group (PVSG), the British Society for Haematology (BSH) and the World Health Organisation (WHO) to diagnose PV and differentiate it from other causes of erythrocytosis. Recently it has been shown that PV is associated with an acquired activating mutation,V617F, of the Janus kinase (JAK)2. *Aims.* We retrospectively assessed the diagnostic information of patients with erythrocytosis of all causes (PV, idiopathic, secondary and apparent) against the BSH criteria. We determined if a diagnosis of PV would have been established by these criteria and whether or not this agreed with the diagnosis made by their clinician. We are determining the JAK2 status of this group. *Methods and Results.* The patient sample was drawn from a clinical database. The records of 77 patients who attend Belfast City Hospital with PV (47 patients) and other causes of erythrocytosis (30 patients) were reviewed and relevant information was recorded. Sufficient data was available to apply the BSH criteria to 64 (PV 36, other 28) out of 77 patients (83%). Thirty-five patients met the BSH criteria for a diagnosis of PV and 29 patients did not. There was agreement with the diagnosis established by the patients clinician in 65 out of 66 cases. Only 1 patient had been diagnosed with PV who did not meet the BSH criteria. This patient met both the WHO and PVSG criteria for PV. To date the JAK2 V617F mutation has been demonstrated in 29 out of 31 tested patients with PV and in 1 of 12 patients with erythrocytosis of other causes. *Conclusions.* We concluded that the BSH criteria for the diagnosis of PV were easily applied, sensitive and specific. Results of V617F JAK2 mutational analysis are consistent with previous findings and support the suggestion that this should be incorporated into the initial evaluation of patients with erythrocytosis.

1449

RESPONSE TO IMATINIB IN A FIP1L1-PDGFR α NEGATIVE HYPEREOSINOPHILIC SYNDROME(HES) PATIENT WITH STROKE AND INFECTIVE ENDOCARDITIS

S. Mitra, O. Kelly, P. Evans, J. O'Dwyer, J. Moroney, P.T. Murphy

Beaumont Hospital, DUBLIN, Ireland

Backgrounds. The Hypereosinophilic syndromes are a rare haematologic disorder characterized by eosinophilia(>1.5×10^9/L) persisting for more than 6 months in the absence of reactive causes. Recently the FIP1L1-PDGFRα Fusion gene has been identified in 50% of cases of HES. This fusion gene is a constitutively activated tyrosine-kinase. Imatinib mesylate is a potent selective inhibitor of several Tyrosine Kinases, including PDGF receptors. *FIP1L1-PDGFRa* fusion gene seems to be another target of this drug.

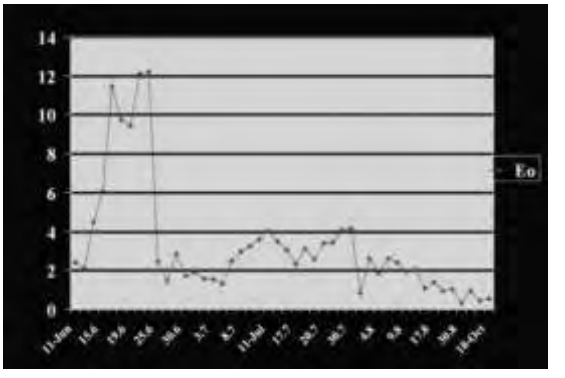


Figure 1. HES-Steroids commenced 25/6/5, Imatinib from 29/7/5.

Case History. We present a 45 year old man ,with background history of allergic rhinitis and asthma, who presented with left sided weakness and slurred speech. A MRI scan showed a right lacunar infarct. A CT scan done after 4 days revealed a large right intra cranial haemorrhage. He also had transoesophageal echocardiogram(TOE) evidence of mitral valve endocarditis which was treated with appropriate antibiotics. His FBC revealed a eosinophil count of 12.Further investigations revealed no evidence of a parasitic infection or drug allergy. As an inflammatory aetiology was initially suspected he was initially commenced on Methyl Prednisolone which was later changed to oral Prednisolone(1 mg/kg). A subsequent Brain Biopsy was negative for vasculitis and bone marrow aspirate/biopsy revealed increased eosinophil precursors but there was no evidence of lymphoma. Molecular studies of marrow aspirate revealed no evidence of clonal T cell rearrangements.There were no metaphases noted on cytogenetic analysis of the aspirate. The aspirate was sent away for detection of the *FIP1L1-PDGFRa* by RT-PCR. His eosinophil count fell to 3.5-4 on oral Prednisolone. A trial of Imatinib was considered appropriate. This was commenced at a dose of 400mg/day. *Results.* A response in the eosinophil count(=0.84) was noted in 3 days. Over the next 2-4 weeks the eosinophil count fluctuated but remained between 0.8 and 2.0 .The Prednisolone was gradually tapered off to zero. *FIP1L1-PDGFRa* came back negative.The Imatinib dose was reduced to 200 mg per day after 3 weeks.When he was discharged ,after about a month later his eosinophil level was 0.78 and his maintenance dose of Imatinib was100 mg/day. Discussion:It's worth noting from this case that a trial of imatinib is worthwhile after all the necessary blood tests have been sent.We note that this patient's eosinophilia responded to steroids but the count came down to near normal levels after 4 weeks of Imatinib. The result of his *FIP1L1-PDGFRa* came back as negative but he was clearly an Imatinib responsive HES. Thus he may have one of the other very rare fusion genes associated with eosinophilia (causing dysregulation of the enzyme Tyrosine Kinase), which respond to Imatinib but are difficult to test in the absence of karyotypic abnormalities.It is not clear whether the high dose steroid contributed to the PCR negativity for FIP1L1-PDGFRα *Conclusions.* Thus Imatinib is very useful therapeutic option in majority of Hypereosinophilic syndromes Early control of eosinophilia may be acheived even in FIP1L1 Negative HES and this may decrease further end organ damage.

1450

THROMBOCYTOSIS. ETIOLOGIC ANALYSIS OF 1688 HOSPITAL PATIENTS

A.K.Z. Zacharof

Hellenic Red Cross Hospital, CHALANDRI, Greece

Background-Aims-Methods : One thousand six hundred eighty eight hospital patients aged 21 to 86 years with thrombocytosis (defined as a platelet count of more than 450.000/cu mm and below 1.000.000/cu mm in 97% of patients) seen in our hospital over a 15-year period, were studied prospectively for etiological diagnosis. *Results.* The causes of thrombocytosis were myeloproliferative diseases (6%), malignancy (17%) post-surgery or experiencing tissue damage,massive acute hemorrhage or thrombotic episodes (19%), infections (34%),chronic inflammation (5%), iron deficiency anemia (10%), miscellaneous disease states as cardiac disease, liver cirrhosis, renal failure etc (9%). Thrombocytosis associated with multiple, simultaneous causative factors was seen in 7.9% of cases. Among all hospital patients with infections, sepsis was associated with higher platelet counts than any other infection ($P < .0001$). Thrombocytosis secondary to infections and malignancy was significantly more common in aged patients. No thrombocytosis-related complications were seen in any hospital patient and none required any specific treatment. Conclusions.Thrombocytosis is a frequent finding in hospital patients. It is due to a variety of etiologic factors and is of significance clinical discriminatory value. It is often due to an acute-phase phenomenon in response to infection, tissue damage, blood loss, or anemia, and is an early sign especially of disseminated, advanced or inoperable malignancy.

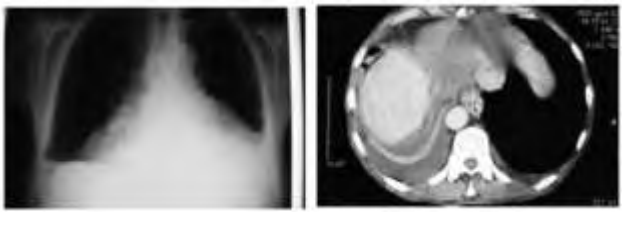
1451

PERICARDIAL EFFUSION IN MYELOPROLIFERATIVE DISORDERS

S. Mitra, P. Evans, P.T. Murphy

Beaumont Hospital, DUBLIN, Ireland

Backgrounds. Extramedullary haematopoiesis is very common in Chronic Myeloproliferative Disorders(MPDs) and the commonest site of extramedullary haematopoiesis is the spleen and the liver.Unusual sites can sometimes be affected leading to haematopoietic tumours surrounded by a capsule of connective tissue. Such sites includes lymph nodes, CNS, skin, pericardium, peritoneum, pleura ovaries,GIT and the lung. Many such cases remain asymptomatoic and may be diagnosed incidentally. However in cases where the pericardium is affected cardiac tamponade may result,requiring urgent intervention (pericardiocentesis). Recurrence may be prevented by minor pericardiectomy. In patients with myeloproliferative disorders and increased cardiac silhouette on X-Ray film,with or without clinical heart failure an echocardiogram is recommended in order to identify a possible pericardial effusion. Treatment with radiotherapy have been shown to be effective. Case History:We report a 68 year old man who recently presented with weight loss, mild anaemia moderate thrombocytosis and leucocytosis.A diagnosis of a Myeloproliferative disorder was made 3 years back but he had been transfusion independant.He has background history of atrial Fibrillation on Warfarin (thus his thrombocytopaemia might result in bleeding while he was on Warfarin). On examination he had 4 cm splenomegaly and 5 cm hepatomegaly. A bone marrow aspirate showed myeloid hyperplasia with no evidence of transformation.Cytogenetics revealed loss of y chromosome (of no significance). FISH for bcr/abl was negative.The trephine biopsy was consistent with a Myeloproliferative Disorder. Thus the diagnosis was MPD (unclassifiable). Five days after admission he developed diarrhoea which was treated empirically with oral Metonidazole after stool samples were sent.



As he was dehydrated, he was commenced on IV fluids and a further 48hrs later he developed bilateral pedal oedema upto his knees. He had clinical evidence of CCF. An ECG showed low voltage complexes and an Echocardiogram revealed a concentric pericardial effusion of 2.46 cm.The right atrium was not collapsing on inspiration.He was transferred urgently to the Coronary Care Unit(CCU) so that urgent pericardiocentesis may be done if he developed cardiac tampomade.He remained stable in the CCU. Serial echocardiograms did not reveal any evidence of increase of the effusion. A CT (thorax/abdomen) showed moderate pleural effusion, pericardial effusion with massive hepatosplenomegaly and mild ascites. At present he has required no intervention for his pericardial effusion. Radiotherapy may be a non invasive option for him in the future. Discussion:There are about 10 Case reports of Pericardial effusion (in Myeloproliferative Disorder) in the literature (MEDLINE search) , 7 of these are in Idiopathic Myelofibrosis, 1 in Essential Thrombocytosis, 1reported in Chronic Myeloid leukemia and 1 in a case of MPD (unclassifiable). *Conclusions.* Myeloproliferative Disorder patients can develop moderate to severe pericardial effusion which can result in dramatic clinical deterioration:hence an high index of suspicion and an early echocardiogram necessary. Close monitoring in CCU will ensure that urgent intervention(pericardiocentesis) can be undertaken if cardiac tamponade develops.

1452

THE INCIDENCE OF THROMBOTIC EVENTS IN CHRONIC MYELOPROLIFERATIVE DISORDERS

M.L. Balea,[1] M. Guran,[1] M.I. Balea,[2] V.E. Leanca,[1] M. Mitri,[1] G. Mihalache[1]

[1]*Clinical Hospital Colentina, BUCHAREST, Romania;* [2]*NIP Marius Nasta, BUCHAREST, Romania*

The thrombophilia due to Chronic Myeloproliferative disorders (CMD) is determinate by modification of rheologic parameters through hyperviscosity, the perturbation on thrombocyte function and the perturbation of the cytokines secretion. The thrombotic events associated to CMD are the result of the thrombophilia feature with the quality of medical care. This is the reason for the analysis of the 136 subjects diagnosed in our Clinics with CMD between 2000 - 2005. We diagnosed in 52 subjects Polycythaemia Vera (PV) (32,8%), in 32 subjects Essential Thrombocythemia (ET) (23,52%), in 26 subjects Agnogenic Myeloid Metaplasia (AMM) (19,1%) and in 26 subjects Chronic Myeloid Leukemia (CML) (19,1%). There were diagnosed thrombotic events in 49,26% subjects: recurrent cerebral thrombosis in 28 subjects (20,58%): 34,46% in PV, 18,75% in ET, 7,79% in CML. Recurrent thrombophlebitis in 8 subjects (5,88%):12,5% in ET, 7,69% in AMM and 3,84% in PV. Superficial thrombophlebitis: 2 subject in ET (6,25%;) central retinal vein thrombosis: 2 subject in CML (7,69%); Disseminated intravascular coagulation: 2 subject in ET (6,25%); Spleen infarction: 2 subject in CML (7,69%); Portal vein thrombosis: 4 subjects (2,94%): 7,69% in AMM and 6,25% in ET; Arterially and capillary thrombosis 8 subjects (5,88%): 11,53% in PV, and 6,25% in ET; Necrotizing purpura: 1 subject in TE (3,125%); Heart infarction: 9 subjects (6,61%): 15,625% in ET, 7,7% in PV; Mesenteric infarction 1 subject in ET (3,125%). The frequency of thrombotic events was 75% in ET, 61,5% in PV, 23,07% in CML and 15,38% in AMM. *Conclusion*. The thrombotic events are an important risk factor in CMD, especially in ET and PV. 75% of thrombotic events developed before the diagnostic of CMD and 25% after this. This impose to facility the access to modern therapy: Erythropheresis, Anagrelide, Glivec (imatinib), α interpheron and to consider the primary and secondary thrombophilia as major risk factor for cardiovascular disease.

1453

WHY WE HAVE NO WORKING SYSTEM OF ELECTRON CASE HISTORY UNTIL NOW? EFFORTS TO DEVELOP THE NATIONAL STANDARD

N. Shklovskiy-Kordi, V. Zingerman, V. Surin

National Center for Hematology, MOSCOW, Russian Federation

The computerized case history system allows the collection of medical information from multiple sources, integrated presentation, fast search, simultaneous availability to several participant of medical process etc.,. It is efficient for patient follow-up, consultation, and transfer as well as for analysis of clinical trials. The subsidiary role of information systems in medical process partly the result of insufficient attention is given to the question of the official status of electronic personal medical records and documents. In the majority of hospitals such systems are used only for preparation and printing of medical documents, which signed by ink, participate in traditional medical document circulation. Case history or research forms of clinical trials shelved in storage and if could to be retrospectively analyzed, only with huge efforts. It seems, that most of pharmacological companies, conducting clinical trails are

not interested in direct collecting data in electron database, because such system able to provide real audit of study procedures and conclusions, practically impossible with paper form collection. Use of the electronic medical document and electronic archives demands to provide: - An invariance and reliability during all period of storage; a regulation of rights of access and confidentiality; personification (an opportunity to define the author and an origin of record at any moment - analogue of the signature on the traditional document). Concerning the traditional medical documentation a lot of normative were developed. Electronic personal medical records needs the development of standards providing their legal status and an effective utilization in medicine and public health services. It's so happened in Russia, that the project 'The National Standard of electron case history' were presented by the National Center for Hematology.

1454

ERDHEIM-CHESTER DISEASE: TREATMENT WITH INTERFERON-α

M.C. Favarin, D.C. Lemos, D.C. Tarquino, F. Chahud, C.F. Mendes, M.I.A. Madeira, C.E.E. Velano, L.C. Palma, L.C.O Oliveira

School of Medicine of Ribeirao Preto, RIBEIRAO PRETO, Brazil

Erdheim-Chester (EC) is a rare non-Langerhans' cell histiocytosis of unknown etiology. This rare illness has a particular tropism for connective and adipose tissues. There are typical radiographical and pathological features, which can lead to the diagnosis, but the clinical spectrum ranges from a focal assymptomatic process to a multisystemic , rapidly fatal, infiltrative disease. The entity is defined by a mononuclear infiltrate consisting of lipid laden, foamy histiocytes that stain positively for CD38, and negatively for CD1a, and S100. The differential diagnosis includes Langerhans' cell histiocytosis, metabolic disorders, and malignancies. The outcome of patients is worse than that for Langerhans' cell histiocytosis with about 60% of patients dead after a mean follow-up of 32 months, whereas only 9% of patients with the latter disorder have succumbed after a median follow-up of 4 years. Corticosteroids, chemotherapy, surgical resection, and radiotherapy have been used to combat EC disease and there is no consensus concerning the best treatment. A recent study showed good outcome of 3 patients with advanced disease but without pulmonary and cardiac involvement treated with interferon-α (IFN-α). The aim of this study is to present an advanced EC case with cardiac, pulmonary, bone, retro-orbital and retroperitoneal fibrosis that showed bad outcome of the cardiac function with IFN-α therapy. We evaluated a 48-year-old woman with a 2-year history of striking exophthalmos. Her previous history included retroperitoneal fibrosis with unknown cause and obstructive renal impairment that led to chronic kidney failure. The ophthalmologic examination revealed a Hertel exophthalmometry measurement of 27mm (normal 12-20mm). Computed tomography of the orbits showed massive infiltration. Retro-orbital mass biopsy was consistent with EC. Prior to treatment the exams showed 46% of heart ejection fraction on the left ventricle, lung function with a mild restrictive ventilation defect and a simetrical involvement of long bones (sclerotic bone lesions in femora, tibiae and radii). INF-_ was started at a dose of 3 x 106 units, subcutaneous, 3 times per week. After one month, the exophthalmometry measurement revealed a reduction of 2mm in the right eye and 1 mm in the left eye. Two months later, the computed tomography of the orbits showed regression of 3 mm in retro-orbital mass bilaterally. Nevertheless, heart ejection fraction was reduced to 26% after the same period of treatment. Although there was an evident improvement of exophthalmos, the patient showed a pronounced decrease of the cardiac function. IFN-α has been widely used in clinical practice as an antiviral, anticancer, and immunomodulatory agent. On the other hand, IFN-α is known to induce adverse effects such as cardiac dysfunction, cardiomyopathy, various kinds of arrhythmias, and sudden cardiac death, although clinical trails which evoke these cardiac events are not documented. This aggravation could be, in part, imputed to the interferon-α therapy. So, the decision of use this therapy in patients with previous cardiac disfunction should be done carefully and a closely heart evaluation can be useful.

1455

COMPARISON OF TWO DIFFERENT TOP AND BOTTOM DEVICES FOR CORD BLOOD VOLUME REDUCTION

M. Solves,[1] V. Mirabet,[1] M. Arnao,[2] D. Planelles,[1] A. Soler,[1] R. Roig[1]

[1]*Transfusion Centre, VALENCIA, Spain;* [2]*Hospital de la Ribera, ALZIRA, Spain*

The major problem with long-term cord blood banking is the required storage space. Red blood cell (RBC) depletion of cord blood (CB) collections not only maximises storage space but have also another advantages like the reduction of the amount of DMSO as cryoprotectant thus reducing the potential side-effects of DMSO and the reduction of side-effects of ABO mismatched or hemolyzed red blood cells. Our cord blood bank performs volume reduction with top and bottom devices. Aims. To compare two different top and bottom devices for the cord blood volume reduction purpose. The obstetrical team evaluated maternal and neonatal pairs during the pre-partum period in the maternity wards collaborating in the cord blood program. Donors signed informed consent before delivery, during last months of pregnancy. The CB units collected in triple bag system were centrifuged in oval buckets at 3000 g for 12 min at 22°C, ensuring that the bags were well supported to prevent disruption of the buffy coat layer. CB collections were separated into plasma, red blood cells concentrate (RBCC) and buffy coat (BC) containing haematopoietic progenitors with two different devices: Optipress II and Compomat G4. A standard protocol programmed into the Optipress II, together with the standard backplate for BC preparation was used to process the CB units (n=27). The programme was set with the following parameters: BC volume of 40 ml, a BC level of 5.5 and a force of 25. Program CB1 in Compomat G4 device was empirically developed to reach a BC volume of 41 ml (n=31). Monitoring the TNC, RBC, CD34+ cells and CFU content in both pre-process and post-process CB units assessed the volume reduction process during the development phase of the study. Results. Table 1 shows the results of the development phase of the study. When the two devices were introduced into routine, lymphocytes recovery (79.6±10.9% for Optipress II and 77.3±7.5% for Compomat G4, $p<0.001$) and red blood cell depletion (55.6±16.1% for Optipress II and 51.4±14.9% for Compomat G4, $p<0.005$) were significantly better for CB units processed with Optipress II. TNC recovery was similar for both methods (78.5±7.8% for Optipress and 78.2±7.2% for Compomat G4, p=ns). Conclusions. Compared to Optipress II, volume reduction with Compomat G4 device allows worse lymphocyte recovery and RBC depletion of cord blood units.

Table 1.

	Optipress II	*Compomat G4*
TCN Recovery (%)	77.5±10.3	79.8±9.3
CD34 recovery (%)	116.6±81.9	85.0±13.6
CFU recovery (%)	93.6±26.8	88.7±41.1

1456

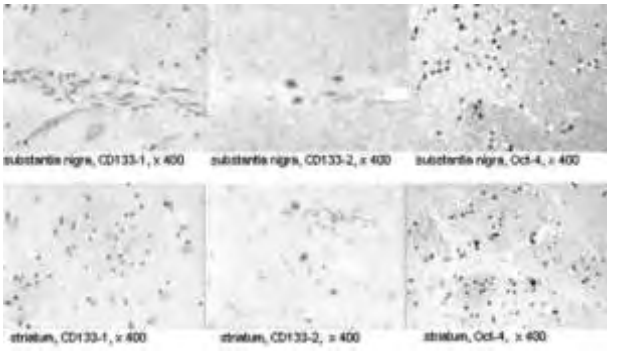
A VERY SMALL POPULATION OF CELLS EXPRESSING THE CD133 HEMATOPOIETIC STEM CELL ANTIGEN EXISTS IN HUMAN ADULT SUBSTANTIA NIGRA AND STRIATUM BRAIN TISSUES

H.T. Hassan, X. Zhai, J.A. Goodacre

Lancashire School Postgraduate Medicine, PRESTON, United Kingdom

Numerous animal studies have demonstrated the presence of neural stem cells in the mammalian forebrain. Recently, the CD133 haematopoietic stem cell antigen has been identified in foetal human brain tissue, human focal cortical dysplasia, premature infants cortex and in paediatric brain tumours. To date, it is unclear whether these stem cells exist in human adult brain tissues. The aim of the present study was to evaluate the presence of CD133 positive cells in the various areas of postmortem human midbrain and hindbrain tissues including the substantia nigra, straitum, medulla and pons. The immunocytochemical staining with anti-CD133 epitope 1 (clone AC133, Miltenyi Biotech Ltd.) and anti-CD133 epitope 2 (clone 293C3, Miltenyi Biotech Ltd.) revealed the presence of CD133 epitope-2 and not epitope-1 in only two sites: *substantia nigra* and straitum in post-mortem brain tissue sections from 4 elderly patients purchased from Medical Solution plc. (Nottingham, UK). The CD133 epitope-2 positive cells were oval prolonged in shape and have size of 130-172 μM. The CD133 positive cells in the substantia nigra were larger than those in the Striatum. The lack of any expression of CD133 epitope-1 in adult brain is in line with previous PCR-studies. Also, we investigated the presence of any expression of the OCT-4 embyonic antigen in the same adult brain tissue sections. Only a smalll population of cells expressing OCT-4 was found only in the same two sites: Substantia Nigra and Striatum as shown in the figures. Since in the substantia nigra of the midbrain, degeneration of dopaminergic neurones is responsible for the debilitating motor dysfunction in patients with Parkinson`s disease. further studies are warrant-

ed to explore the theurapeutic role of these unique CD133 positive cells residing in the substantia ngra and striatum of the adult human brain.



1457

INDUCTION OF HLA-DR AND CD33 EXPRESSION ON GRANULOCYTES AFTER GM-CSF AND G-CSF ADMINISTRATION

A. Kousoulakou,[1] G. Paterakis,[2] E. Terpos,[3] J. Meletis[4]

[1]*Onassis Cardiac Surgery Center, ATHENS, Greece;* [2]*Dep. of Immunology G. Genimatas Hospital, ATHENS, Greece;* [3251]*General Airforce Hospital, ATHENS, Greece;* [4]*Univercity of Athens, Laiko Gen. Hosp., ATHENS, Greece*

Backround: The effect of granulocyte-macrophage colony stimulating factor (GM-CSF) and granulocyte colony stimulating factor (G-CSF) on granulocytes function and phenotype is complicated and depends on the dose, the route and the way (bolus, continuous) of growth factor administration. There are also differences between *in vitro* and *in vivo* effects. *Aim*: The aim of this study was to evaluate the effect of GM-CSF and G-CSF on granulocyte phenotypic expression. *Methods.* We determined the phenotypic expression of granulocytes obtained from peripheral blood of 29 patients with hematological malignancies (7 patients had AML, 7 ALL, 4 CML, 5 non-Hodgkin's lymphomas, 2 myeloma, 1 aplastic anemia, and 3 MDS) and 7 patients with solid tumors. Blood samples were collected on the 1st and 2nd day of growth factor administration, as well as on days 5-20 after the final day of growth factor injection. Phenotypic analysis was performed by the alkaline phosphatase (APAAP) immunocytochemical technique, using a wide panel of monoclonal antibodies. In addition, the in vitro effect of GM-CSF in short term cell cultures was evaluated in 2 cases with AML, 2 with solid tumors and in 2 healthy individuals. Peripheral blood cells were suspended in RPMI with and without FBS 10%, with and without the addition of GM-CSF (consentration: 0.1 ng/mL). Phenotypic analysis was performed using both APAAP and Flow Cytometry (FC), in cells obtained before the suspension (T0h) and after 24h of incubation (T24h). *Results.* High percentage of granulocytes was positive for HLA-DR (Ia) and CD33 after GM-CSF or G-CSF administration (Table 1).

Table 1. Percentage (%) of Ia + and CD33 + granulocytes.

AML	25-100	10-95
ALL	2-83	10-90
CML	6-40	15-80
MDS	0-75	20-100
NHL	15-90	30-98
MM	0	85
AA	10	70
Solid Tumors	10-60	30-90

In most cases those antigens were expressed on granulocytes from the first day of GFs' administration and they were preserved on their surface even 20 days after the final day of GF injection. *In vitro* tests showed induction of HLA-DR on granulocytes after incubation with GM-CSF (Table 2). *Conclusions.* According to our data, administration of growth factors induces the circulation of HLA-DR+ granulocytes in peripheral blood. In vitro tests also confirm this finding. In addition, the results from in vitro tests indicate that GM-CSF directly affects granulocytes, inducing synthesis and expression of the antigen-presenting molecule HLA-DR. Still remains to be proved whether this expression has any functional antigen-presenting role. CD33 is a marker of immaturity and has a regulatory effect in the maturation of myeloid cells, the monocytes/macrophages function and the production of dendritic cells. Its expression on high percentage of granulocytes could be due to: (a) increased mobilization of granulocytes from bone marrow due to CFs' effect; (b) high rate of differentiation, resulting in preservation of immature markers on granulocytes surface; and (c) possible reactivation of CD33 genes due to cytokines effect.

Table 2. Percentage (%) of Ia + and CD33 + granulocytes *in vitro* tests.

		T0h		T24h	
		RPMI	RPMI+GM-CSF	RPMI+FBS	RPMI+GM-CSF+FBS
Healthy	0-2	0	5-20	5-10	20-30
AML	2-5	0-10	15-35	10	3
Solid tumors	5	5-20	20	10-25	20-30

1458

SEVERE AZATHIOPRINE-INDUCED BONE MARROW APLASIA IN THIOPURINE S-METHYLTRANSFERASE DEFICIENT PATIENT WITH CROHN'S DISEASE

B.M. Zdziarska, M. Kurzawski, E. Wierzbicka-Paczos, M. Chosia, M. Drozdzik

Pomorska Akademia Medyczna, SZCZECIN, Poland

Thiopurine S-methyltransferase (TPMT) is an enzyme that catalyses S-methylation of thiopurine drugs, which activity is genetically determined. TPMT-deficient patients are at risk of toxicity after standard doses of thiopurine drugs. There is a large interindividual variability of TPMT activity, mainly due to genetic polymorphism. The three variant alleles: TPMT*2, *3A and *3C are responsible for over 95% cases of lower enzyme activity. *Case Report*: A 49 year-old male patient diagnosed with Crohn's disease at the age of 45 was administered azathioprine (AZA) at a daily dose of 2.5 mg/kg. After a month of treatment he developed severe bone marrow aplasia, confirmed by bone marrow biopsy and was hospitalized. Genotyping for TPMT polymorphism (*1, *2, *3A, *3B and *3C) was performed by PCR-based methods and revealed variant homozygous genotype (TPMT*3A/*3A), determining deficiency of TPMT activity. After AZA withdrawal from treatment the patient's blood cell count's started to normalize. He was prescribed methylprednisolone at a daily dose of 24 mg and finally disposed home 17 days afterwards AZA withdrawal, with the following blood parameters: RBC - 2.99 T/L, WBC - 14.9 G/L, PLT - 111.0 G/L. This observation is in concordance with the previous reports, indicating that myelosuppression in TPMT-deficient patients treated with a standard dosage of thiopurines occurs on average 1 month after initiation of treatment. Available data shows, that TPMT-deficient Crohn's disease patients can be efficiently and safely treated with 5-15% of standard AZA dose (0.16-0.29 mg/kg) or AZA should be replaced by other medication, otherwise it involves severe myelosuppression. In the present report a clear coincidence of AZA administration and severe myelosuppression is documented. In the view of lack of any other potential factors, which might contribute to the observed myelosuppression it can be concluded that accumulation of toxic thioguanine nucleotides in a TPMT deficient patient, was responsible for the observed bone marrow aplasia. For the reasons given above, evaluation of TPMT polymorphism in patients treated with thiopurine drugs should be mandatory in order to optimize therapy.

1459

MUTATION SCREENING IN THE HUMAN ε-GLOBIN GENE USING SINGLE STRAND CONFORMATION POLYMORPHISM ANALYSIS

A. Papachatzopoulou,[1] P. Menounos,[2] C. Kolonelou,[1] G. Patrinos[3]

[1]*University of Patras, School of Medicine, PATRAS, Greece;* [2]*Nursing Military Academy, ATHENS, Greece;* [3]*Erasmus MC, Faculty of Medicine, ROTTERDAM, Netherlands*

Backgrounds. The human ε-globin gene is indispensable for primitive human erythropoiesis. Embryonic ε-globin gene is expressed at high levels early in development in the yolk sac, producing the ε-globin chain, which participates in the formation of the abovementioned Hb tetramers. Although there are over 1,200 different mutant alleles, identified in the human α-, β-, γ- and δ-globin genes, there are no nucleotide

changes in the human ε-globin gene reported to date, rather than a single nucleotide polymorphism (SNP), located at the 5' regulatory region of the gene. *Aims.* To develop a non-radioactive single strand conformation polymorphism (SSCP) approach to screen the human _-globin gene and its regulatory regions for possible mutations and single nucleotide polymorphisms in normal adult subjects, in order to determine those genomic regions, which are dispensable for its proper regulation and function. *Methods.* Peripheral blood was collected from 60 unrelated normal male and female donors, whose age ranged between 25-50 years. Informed consent was obtained prior to the study. Genomic DNA was extracted from peripheral blood leucocytes. Human ε-globin gene coding and regulatory regions were amplified in 5 consecutive fragments, using 25 pmoles of each primer (primer sequence available on request). PCR products were then analyzed, using a non-radioactive (silver-staining) single strand conformation polymorphism (SSCP) analysis. Where needed, temperature has been adjusted (from 4-8°C, increment: 1°C) to improve resolution. DNA sequence analysis was performed using automated fluorescent DNA sequencer (ABI PRISM 310 Genetic Analyzer, Applied Biosystems, CA, USA). *Results.* Selection of the fragments was done on the basis of analyzing the entire coding and regulatory regions of the ε-globin gene in addition to the majority of intronic sequences. Heterozygous and homozygous cases for the 5'ε/HincII SNP were analyzed as positive controls to ensure optimal resolution and detection of the expected nucleotide changes. Apart from the abovementioned SNP in the expected frequencies for the Hellenic population, our mutation screening approach of 120 normal chromosomes from adult individuals yielded no other nucleotide change in all samples in the region analyzed. DNA sequencing of 10 randomly selected cases in all 5 fragments confirmed the presence only of the wild-type sequence. A reminiscent of this situation is the human ε-globin genes, which are also mutation-free in their proximal promoter regions. *Conclusions.* This observation suggests that nucleotide changes in the human ε-globin gene are most likely incompatible with normal erythropoiesis and proper embryonic development. The possibility that mutations or SNPs are present inside the 562bp region of intron II, which has not included in our experimental design, cannot be ruled out, although it is less likely as this region is also mostly unaltered in the rest of the globin genes studied.

1460

LONG-TERM FOLLOW-UP OF PATIENTS SUFFERED FROM SEVERE APLASTIC ANEMIA-TREATED WITH IMMUNOSUPPRESSIVE THERAPY AND/OR STEM CELL TRANSPLANTATION

L.J. Tukic, D. Stamatovic, O. Tarabar, V. Glavicic, M. Elez, L.J. Simic, M. Malesevic, S. Marjanovic

Military Medical Academy, BELGRADE, Serbia and Montenegro

Backgrounds. Stem cell transplantation (SCT) from a HLA-identical fully matched sibling donor (MSD) is the best treatment option for severe aplastic anemia (SAA). Patients without suitable MDS should be treated with immunosuppressive therapy (IST). *Patients and Methods.* between 1/1985 and 2/2006 41 patients (pts) with newly diagnosed SAA were treated either with SCT from MSD (13 pts) or with IST (28 pts). There were performed 15 allogeneic SCT in 13 pts. All donors were HLA-identical sibling (1 donor was identical twins). Source of stem cells was bone marrow in 12 (two with second transplants) and peripheral stem cell in 3 SCTs. Conditioning regimens were based on cyclophosphamide (CY) with antithymocyte (ATG) in 10 and Flud with CY and ATG in 3 SCTs. 20 pts received combined IST with ATG or ALG (antilymphocyte globuline), cyclosporine A and steroids and 12 pts ATG with steroids (from which 4 were splenectomized). The median interval from diagnosis to ISH was 30 days (range 23 to 510). *Results.* engraftment was documented in 12 pts with allogeneic SCT (1 pts died without engraftment). One patient developed acute GvHD grade 3-4 and died on 48 days, the other pneumonitis interstitialis (CMV+) and died on 60 days and the third rejected allograft after 7 months and died 8 days following the second SCT. Up February 2006, 8 of 12 pts (66.7%) are alive with sustained engraftment. Median survival from SCT is 64 months (range 14 to 192). With IST at 23 of 28 patients (82.1%) achieved a response (7 had two or tree cycles IST). Two pts relapsed 1 year after IST. Four pts from IST group died, major causes of death were infection and hemorrhage. Overall survival in the IST group is 64.3% (18/28 patients) after a median follow up of 94 months (range 9 to 240). At two pts from IST group (11%) diagnosed MDS/AML after 87 months (range 72 to 102). *Conclusion*: Our results confirm significant improvement in SAA patients outcome with induction modern front line therapy including allogeneic SCT and IST during last two decades.

1461

OUTCOME OF CHILDREN WITH APLASTIC ANEMIA IN A DEVELOPING COUNTRY. A SINGLE CENTRE EXPERIENCE

M. Serban,[1] M. Bataneant,[2] S. Arghirescu,[2] E. Boeriu,[2] C. Jinca,[2] R. Firescu,[2] G. Doros,[2] R. Badeti,[2] D. Lighezan[2]

[1]*University of Medicine 'V. Babes', TIMISOARA, Romania;* [2]*University of Medicine 'V. Babes', TIMISOARA, Romania*

Background. Aplastic anaemia (AA) is a heterogeneous clinical syndrome, representing a serious challenge for a health system confronted with shortages and with limited experience in bone marrow transplantation. *Aims*. Given these conditions of treatment we sought to evaluate outcomes of patients with AA treated in our centre. *Methods*. We retrospectively analyzed the records of 28 patients consecutively admitted in our centre in the period from 1995 to 2005; their mean age was 10.5 years (3 months 22 year) and the sex ratio male/female was 1.33. They all met the criteria for AA: 2- non severe, 2 -severe and 24 -very severe form. For all patients a diagnostic workup had been performed consisting of: quantitative serum immunoglobulin panel, flowcytometry study of lymphocytes, anti-nuclear and anti-DNA test, viral hepatitis, cytomegalovirus, Epstein-Barr virus, HIV-1 and 2 serologies, bone marrow aspirate and biopsy, including histologic and cytogenetic analyses. Three patients fulfilled the criteria for hereditary AA (Fanconi anemia-1, Dyskeratosis congenita-1, familial autosomal dominant form -1 case), 6 cases were assessed as postinfectious forms (hepatitis and HIV infection), 1 case was connected with selective IgA deficiency and 18 were idiopathic forms. *Results.* The treatment consisted of: corticosteroid therapy ± G-CSF (4 cases), androgen preparations (12 cases) and cyclosporine A (CsA) (17 cases); 10 patients received a standardized regimen of antithymocyte globuline (ATG) and CsA; one patient with very severe form was transplanted with related HLA-compatible marrow. Most of patients (17-60.7%) died: 8 with severe sepsis, 8 with bleeding accident and 1 developed a fatal myeloblastic leukemia. The unfavourable outcome characterized 3 patients with hereditary form (except the patient with diskeratosis congenita), all patients with postinfectious AA and the patient with IgA deficiency. 12 cases with idiopathic AA survived, 8 of them with very severe form, treated during the first 2-4 months of disease with ATG + CsA. *Conclusions*. AA remains in our experience the hematological disease with the worst prognosis. To assure the accessibility to allogeneic bone marrow transplantation and to appropriate immunosuppressive therapy is our mandatory future task.

1462

ACUTE LEUKEMIA AND MYELODYSPLASIA REVEALING FANCONI ANEMIA: REPORT OF 6 CASES.

M. Laatiri,[1] K. Zahra,[1] M. Bedoui,[2] M. Kortas,[1] A. Kelaf[1]

[1]*Hopital Farhat Hached, SOUSSE, Tunisia;* [2]*Hopital Farhat Hached, SOUSSE, Tunisia*

Fanconi anemia (FA) is a rare autosomal recessive disease characterized by progressive pancytopenia, congenital malformations and predisposition to myelodysplasia (MDS) and acute myeloid leukemia (AML). FA is rarely revealed by AML or MDS. We report 6 cases of patients unknown before with AF and who develope AML and MDS. The ages of patients ranged from 3 to 25 years. The mean age at diagnosis was 11 years. Malformations were present in two cases and consisted of skeletal malformations. Abnormal skin pigmentations were present in 5 cases. AML was noted in 4 cases and MDS in 2 cases. The diagnosis of FA had been proven by chromosome breakage analysis. Cytogenetic analysis showed monosomy 7 in 3 cases and del 6p in one case. Chemotherapy was delivered only in 2 cases. The outcome was unfavourable with death in 5 cass. This study suggest to perform systematically a cytogenetic analysis to diagnose FA in childhood AML in tunisian population, which is characterized by its heterogenous ethnic background and by a high rate of consanguinity.

1463

PRE-TRANSPLANT BONE MARROW MICROENVIRONMENT PLAYS AN IMPORTANT ROLE FOR ENGRAFTMENT AND TRANSPLANTATION OUTCOME IN NON-MYELOABLATIVE STEM CELL TRANSPLANTATION

Y.H. Min, Y.R. Kim, J.Y. Cho, J.S. Kim, I.H. Park, J.W. Cheong

Yonsei Universtiy College of Medicine, SEOUL, South-Korea

Backgrounds. It has been suggested that both bone marrow (BM) microenvironment and hematopoietic stem cells play important roles

for engraftment and immuno-hematopoietic recovery after myeloablative hematopoietic stem cell transplantation. However, the relevance of BM microenvironment implicated in the engraftment and transplantation outcome after non-myeloablative stem cell transplantation (NST) has not been thoroughly evaluated. *Aims.* In this study, we tried to evaluate the relevance of BM microenvironment implicated in the engraftment and transplantation outcome after non-myeloablative stem cell transplantation (NST). *Methods.* We evaluated quantitatively the pre-NST BM microenvironment with respect to BM cellularity, BM fibrosis, and presence of multi-lineage dysplasia. In addition, for estimating the iron overloading status, we measured the serum ferritin level simultaneously. A total of 51 patients received an allograft using a fludarabine/busulfan NST regimen incorporating alemtuzumab from a sibling (n=39) or unrelated donor (n=12). The underlying disease were as follows: acute myeloid leukemia in 21, severe aplastic anemia in 7, myelodysplastic syndrome in 7, multiple myeloma in 6, chronic myeloid leukemia in 4, and non-Hodgkin's lymphoma in 3 cases. The median age of the recipient was 46 years (range, 18 to 67 years). Although all patients received more than 2×10^6/kg of CD34-positive cells during allografting, five patients (9.8%) had graft failure. Pre-transplant poor BM microenvironment was arbitrarily defined as having one of the following parameters; BM cellularity < or = 20%, BM fibrosis (grade = or > 3), presence of myelodysplastic feature (= or > 2 lineages), and serum ferritin level = or > 1,000 microg/L. Neither of these four parameters was independently associated with graft failure after NST, although there was a higher trend of graft failure in the patients with serum ferritin = or > 1,000 μg/L (5/26, 19.2%) compared to the cases with serum ferritin < 1,000 microg/L (0/27, P = 0.051). Disease-free survival rate was significantly lower in the cases with iron overloading before NST (p=0.019). When the patients were divided into two groups according to the sum of each parameter (< or = 1 vs > or = 2), the patients with more than 2 parameters showed higher probability of graft failure (5/24, 20.8%) compared to cases with less than 2 (0/27, p=0.018). Disease-free survival rate was also significantly lower in the patients with more than 2 parameters (p=0.041). However, there were no significant differences between two groups in the incidence of acute graft-versus-host disease (GVHD) (> or = grade 2) and chronic GVHD. Taken together, we suggest that pre-transplant BM characteristics tentatively reflecting microenvironment function provide valuable informations predicting the engraftment and transplantation outcome, including disease-free survival, in NST settings.

1464

SYMPTOMS IN LONG-TERM SURVIVORS OF CHILDHOOD BLOOD CANCER AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT)/HEMATOPOETIC STEM CELL TRANSPLANTATION (HSCT)

N. Stancheva,[1] L. Zubarovskaya,[1] E. Semenova,[1] M. Ovsyannikova,[1] T. Nikitina,[2] T. Ionova,[2] A. Novik,[3] B. Afanasiev[1]

[1]*St. Petersburg State Medical University, ST.PETERSBURG, Russian Federation;* [2]*Multinational Center of QoL Research, ST. PETERSBURG, Russian Federation;* [3]*National Pirogov Medical Surgical Center, MOSCOW, Russian Federation*

Background. Allogeneic BMT/HSCT improves outcomes in children with blood cancer. However late effects in long-term survivors after BMT/HSCT are understudied. *Aims*. In this connection the aim of our study was to assess symptoms in long-term survivors of childhood blood cancer after allogeneic BMT/HSCT. Patients and Methods. Eighteen survivors were evaluated at 1-13 years (median, 3 years) after allogeneic BMT/HSCT for acute leukemia (15), chronic leukemia (2) and myelodysplastic syndrome (1). Median age at transplantation was 13.5 yrs (range 2 - 21), girls/boys - 11/7. Acute or chronic graft-versus-host-disease (GVHT) after BMT/HSCT was observed in 12 survivors. For symptom assessment NJ Children Cancer Symptom Inventory and MD Anderson Symptom Inventory were used in the group younger than 18 yrs at the time of the survey (n=11) and in the group 18 yrs and older (n=7), respectively. The Smiley Faces Scale was used for symptom assessment in five-year old girl. Results. All the survivors experienced at least one symptom. Twelve survivors (67%) had moderate-to-severe symptoms. The most prevalent symptoms were fatigue and pain (90% survivors); 25% survivors presented moderate-to-severe pain or fatigue. The other prevalent symptoms were lack of appetite (78% survivors, 21% - moderate-to-severe level), sadness (72% survivors, 31% - moderate-to-severe level) and sleep disturbance (56% survivors, 10% - moderate-to-severe level). Other symptoms were shortness of breath, drowsiness, nausea and vision problems. Nine survivors had at least two moderate-to-severe symptoms. Two of them experienced 5 moderate-to-severe symptoms. Among those with moderate-to-severe symptoms 8 survivors experienced acute/chronic GVHD. Conclusions. Our findings demonstrate that more than half of childhood blood cancer survivors experience different pronounced symptoms in long-term period after transplantation. This confirms the importance of symptom monitoring in order to improve/preserve quality of life in long-term survivors of childhood blood cancer after allogeneic BMT/HSCT.

1465

INCIDENCE OF HLA ANTIBODIES IN ALLOGENEIC STEM CELL RECIPIENTS

U. Posch, S. Ulrich, W. Zinke-Cerwenka, S. Macher, K. Schallmoser, G. Lanzer

Medical University of Graz, Austria, GRAZ, Austria

The presence of patient-anti-donor HLA alloantibodies can increase the risk of graft rejection in allogeneic stem cell transplantations and a positive crossmatch against donor lymphocytes may be a predictor for graft failure. Therefore evaluation of patients` sera for HLA antibodies prior to transplantation is routine in most centres. We additionally focussed on the development of HLA antibodies after HLA fully matched compared to partially mismatched stem cell transplantations and on consequential clinical complications. Sixteen patients who were transplanted with HLA 12/12 matched and 12 patients transplanted with one antigen mismatched allogeneic stem cells were screened for HLA class I and class II antibodies by ELISA based methods at the time of registration, prior to and in monthly intervals subsequently to transplantation. Donors were tested for HLA antibodies once prior to transplantation. B- and T-cell crossmatches based on complement dependent cytotoxicity (CDC) were done in 18 cases, depending on the availability of sufficiently viable donor cells. The mean observation period was 84 (28'202) days post transplantation. Two patients and one donor had HLA antibodies before transplantation, which were not directed against transplanted or host antigens respectively, as these transplantations were fully matched. All lymphocytotoxic crossmatches were negative. Preformed antibodies were detectable up to day +60 after transplantation. Five patients developed de novo HLA alloimmunization between day +14 and day +112. Three of them had received fully matched and two got HLA mismatched grafts. All alloantibody specifities were unrelated to host or graft HLA antigens. Relaps was reported on 6 of the not immunised and one of the immunised patients. There was no association between the development of HLA antibodies and acute or chronic GvHD. The only patient who rejected his graft had no HLA alloantibodies at all. Among our patients HLA antibodies did not raise a problem in transplantation schedule. Nevertheless we routinely go on evaluating all patients for HLA antibodies whenever a donor search is started in order to define unacceptable HLA mismatches. Immediately before HLA mismatched transplantations patients and donors are screened for antibodies against non shared HLA antigens, as donor cells of sufficient viability for crossmatching are not always available. After stem cell transplantations graft-host recognition did not seem predominantly responsible for alloimmunisation in our patients and the development of HLA antibodies was no clear predictive factor for GvHD, relaps or graft rejection.

1466

ALLOGENEIC STEM CELL TRANSPLANTATION FROM A DONOR WITH MOSAIC TURNER SYNDROME

K.M. Manola,[1] C. Sambani,[2] A. Symeonidis,[3] N. Zoumbos[3]

[1]*NCSR Demokritos, ATHENS, Greece;* [2]*NCSR Demokritos, ATHENS, Greece;* [3]*University of Patras Medical School, PATRAS, Greece*

Backgrounds. Turner syndrome (TS) is a genetic disorder affecting 1/2000-2500 liveborn females caused by the complete or part absence of one of the X chromosomes, frequently accompanied by cell line mosaicism. In rare cases of stem cell transplantation (SCT) the only available HLA-matched donor could be a female with mosaic Turner syndrome. As disturbances in the immune system have been detected in TS, X0 lymphocytes may show an increased sensitivity to immunosuppressive therapy throughout the post-transplant period, possibly compromising the patient's immune capacity. *Aims.* The aim of this study is to report a case of allogeneic SCT from a donor with mosaic TS in order to evaluate the outcome of this transplant. *Patient and Results.* A 47-year-old male with AML-M1 received a peripheral blood SCT from his 54-year-old 1 HLA-A antigen matched sister in whom the cytogenetic analysis revealed a constitutional mosaic Turner syndrome

(46,XX[18]/45,X0[7]). The patient was conditioned with the classical Bu-Cy regimen and rescued with 5.3 million/kg of body weight allogeneic CD34+ peripheral blood stem-cells. He received IV cyclosporine and four doses of methotrexate as GVHD prophylaxis, but on day +11 he manifested a hyperacute cutaneous and oral mucosa GVHD grade II. On day+16 the patient engrafted the WBC completely, while platelets engrafted earlier, on day+13. One month later, he manifested post-transplant thrombotic thrombocytopenic purpura. Cytogenetic analyses of the patient revealed the complete donor karyotype 46,XX/45,X0 two times after transplantation. Chimerism study was 99.5% of donor type. On day +97 he manifested an extensive chronic cutaneous GVHD grade III, with conjuctival and oral involvement. The post-transplant course was further complicated by the development of diabetes mellitus, severe osteoporosis with a pathologic smash of the L2 vertebra and CMV reactivation. Nineteen months later, the patient is in fairly good general condition. He has limited cutaneous GVHD, suffering mainly from his orthopedic problem. *Summary/Conclusions.* Our patient manifested osteoporosis, diabetes mellitus and conjuctival defect after SCT from a mosaic TS. Osteoporosis is a rare phenomenon in male patients after SCT, while it is very common in adults with TS who show a reduction in bone mass and an increased risk of fractures. Diabetes mellitus and ophthalmic disorders are also uncommon after SCT. On the contrary, diabetes mellitus is 2-4 times more frequent in TS compared with the general population and ophthalmic problems are seen in 63% of women with TS. As it is has been accepted, dosage of specific genes located on X chromosome in X0 cells are responsible for such abnormalities in TS. Therefore, the X0 cells could have pathologic consequences in the recipient, especially under immunologic stress.

1467

LEVELS OF METALLOPROTEINASE-9 (MMP-9) IN PATIENTS WITH POLYCYTHEMIA VERA

S. Theodoridou,[1] T. Vyzantiadis,[2] V. Perifanis,[1] E. Mandala[1],
I. Venizelos,[1] S. Vakalopoulou,[1] E. Leukou,[1] E. Vlachaki[1],
A. Papadopoulos,[3] V. Garipidou[1]

[1]*Hippokration Hospital, THESSALONIKI, Greece;* [21]*st Dep. of Microbiology, Medical School, THESSALONIKI, Greece;* [3]*AHEPA Hospital, THESSALONIKI, Greece*

Chronic myeloproliferative disorders are characterized by the progressive remodeling of the bone marrow stroma during angiogenesis and the fibrotic tissue deposition. Angiogenesis shares common mechanistic steps between the cells and the extracellular matrix including proteolysis. The family of metalloproteinases (MMPs) and their inhibitors play a serious role in this proteolysis and their balance determines whether the tissue remodeling goes towards matrix breakdown or increased fibrosis. Matrix metalloproteinase-9 (MMP-9) is produced in haematopoietic tissue by monocytes and mature granulocytes and is stored in the gelatinase granules while pro-MMP-9 is also stored in platelets and acts as an endogenous angiogenic factor. In order to investigate the role of MMP-9 as an angiogenic factor in Polycythaemia Vera (PV) and because only one report exists in the literature showing elevated levels of MMP-9 in the plasma of 17 patients with PV, we investigated serum levels of MMP-9 in 38 polycythaemic patients. A total of 38 patients with PV (18 males and 20 females) with a mean age of 56,1±15,54 (m ± SD) years (range 24-81) were included. Twenty five patients were managed with phlebotomy, four received hydroxyurea, eight were managed with hydroxyurea and phlebotoby and one was treated with interferon. Three had clinically detected spleen enlargement. The control group consisted of 16 healthy subjects (mean age 55,3±6 years). The age difference between the two groups was not statistically significant (p=0,82). Serum levels of total MP-9 were measured by a commercial quantitative sandwich enzyme immunoassay. Serum MMP-9 concentrations did not differ among polycythemic patients and the control group (316±249 pg/ml and 446,2±290,3 pg/ml, respectively, p=0,086). In the patient group and in the control group we found no statistically significant correlation between serum MMP-9 levels and platelet counts, haemoglobin, WBC counts and age. No difference was found between patients on different therapeutic regimens. In conclusion the present report demonstrates no difference among polycythaemic patients and healthy individuals in concern to MMP-9 serum levels in a large group of patients. Future studies are needed to investigate whether serum markers for collagen metabolism reflect the disturbed balance of matrix synthesis and proteolytic degradation that exists in neoangiogenesis that characterizes polycythaemia vera and myeloproliferative disorders.

1468

ELEVATED TNF-α AND LDH WITHOUT DISTURBANCE IN PARATHORMONE ASSOCIATED WITH DIFUSE OSTEOLYTIC LESIONS IN LEUKEMIC TRANSFORMATION OF MYELOFIBROSIS

V. Jurisic,[1] T. Terzic,[2] S. Pavlovic,[3] N. Colovic,[4] D. Sefer,[4] M. Colovic[4]

[1]*University of Kragujevac, KRAGUJEVAC, Yugoslavia;* [2]*Institute of Pathology, BELGRADE, Serbia and Montenegro;* [3]*Institute for Molecular Biology, BELGRADE, Serbia and Montenegro;* [4]*Institute of Hematology, BELGRADE, Serbia and Montenegro*

Backgrounds. Myelofibrosis is a clonal myeloproliferative disorder characterized by splenomegaly, abnormal deposition of collagen in the bone marrow, extramedullary haematopoiesis, dacriocytosis and leukoerythroblastic blood smear. Development and sustainment of fibrosis are mediated by complex network of several cytokines. These cytokines mainly include transforming growth factor α, basic fibroblast growth factor, vascular endothelial growth factor, platelet factor 4, calmodululin and tumor necrosis factor α, TNF-α. *Aims.* Based on role cytokines in myelofibrosis, we present an atypical case of leukamic transformation in myelofibrosis associated with diffuse osteolytic lesions and extremely elevated sera TNF-α and LDH without disturbance in parathormone in a 49-year-old female that firstly developed malaise and abdominal pain at first visit. *Results.* The laboratory analyses showed decrease in hemoglobine, platelets and presence of erytroblast and dacriocytes in peripheral blood. Cytological examination disclosed hypocellularity with the presence of all cell lines without increased blasts cells. Bone marrow biosy disclosed hypocellularity, presence of all cell lineage and bone marrow reticulin and collagen fibrosis. A diagnosis of myelofibrosis was established. After 3 years, her condition deteriorated with malasia and bone pains. Physical examination showed pale skin and mucous membranes with enlarged spleen 270 mm in diameter. The laboratory analyses showed Hb of 54 g/l, WBC of 8.0×10^9/L, platelets of 122×10^9/L, with myeloblasts 39%, myelocytes 7%, metamyelocytes 1%, bands 6% segmented neutrophils 18%, eosinophils 1%, lymphocytes 22%, monocytes 6%, and 13 erythroblasts/100 leukocytes. The biochemical analyses showed extremely elevated sera LDH activity (1339 U/l). Bone marrow aspirate was hypocellular with 72% of blasts mostly with characteristics of myeloblasts and more than 20% of monoblastic type. Cytochemical staining with myeloperoxidase showed that 30% of blasts were positive, and 25% of blasts were α-naphtol-esterase positive. The cytological finding was in accordance with FAB M4 type of acute leukemia. The immunophenotyping of the peripheral blood cells expressed HLA-DR (74.96%), CD34 (77.99%), CD13 (60.36%), CD33 (42.60%), CD14 (39.89%), CD4 (42.40%) markers. Cytogenetic examination of bone marrow cells showed inversion of chromosome 16 [46,XX, inv(16)(p13q22)]. RT-PCR studies/MYH11 fusion geneα confirmed cytogenetic finding and revealed the CBF transcript. PCR analysis disclosed the presence of FLT3 Asp835 mutation. Retrospective analyses of extracted DNA from bone marrow histological specimen at the time of diagnosis, showed that there no presence of FLT3 mutations. X-ray showed the presence of diffuse osteolyic lesions in the pelvis, long bones as well as in vertebra bodies. The global skeletal scintigraphy documented diffusely increased accumulation of the radiopharmaca. The values of parathormon in the sera and supernatants of cultured blast cells were normal. TNF-α determined by sensitive LDH release assay, was extremely increased (1421 pg/ml) in comparison to control values of 700 pg/ml. Summary / Conclusions. We postulated that elevated TNFα can be reason for lytic bone lesions, accompanied with high sera LDH activity indicating high bone turnover. Also continuously elevated TNF-α can contribute for developing of the leukemia growth in this patient, as endogenous promoter.

1469

FIP1L1/PDGFR-α- NEGATIVE CHRONIC EOSINOPHILIC LEUKEMIA SUCCESSFULLY TREATED WITH IMATINIB CASE REPORT

T.S. Szotkowski, Z. Pikalova, J. Strasilova, B. Katrincsakova,
J. Hanzlikova, E. Faber, M. Jarosova, K. Indrak

University Hospital, OLOMOUC, Czech Republic

Background. Recognition of tyrosin kinases contribution to pathogenesis of idiopathic hypereosinophilic syndrome/chronic eosinophilic leukemia (IHES/CEL) and imatinib treatment tended to rapid improvement in prognosis of significant part of IHES/CEL patients. Imatinib is especially effective in *FIP1L1/PDGFR-α* (F/P) positive patients. Approximately 40% of responding patients lack the F/P fusion gene, suggesting other tyrosinkinase influence. *Patient and methods.* Authors describe case

report of IHES patient diagnosed in 1998. Serious organ damage developed during 2005 and required treatment initiation. Corticoids were quite ineffective as well as cyclosporin A was. Cytogenetic examination of bone marrow cells revealed in 49% of examined metaphases karyotype 45,X,-Y. Fluorescence in situ hybridization (FISH)-based strategy was used to detect F/P in bone marrow cells. Using bacterial artificial chromosome (BAC) probes fusion of F/P was not revealed. Considering the standard treatment ineffectivity, disease progression and clonal hemopoesis finding imatinib treatment was started. Imatinib in dose of 100 mg daily was administered despite the F/P negativity. *Results*. Eosinophils fully disappeared after 6 days of the therapy. Complete hematologic remission was achieved after 2 weeks. Cytogenetic response will be assessed after 3 month treatment. *Conclusions*. The case of IHES/CEL patient with the karyotype 45, X, -Y, F/P-negative but imatinib-sensitive has not been published yet. Identification of imatinib-sensitive target structure responsible for the disease development is a challenge to future research.

Funding: Supported by the grant of the Ministry of Education of the Czech Republic (MSM 6198959205).

1470

MIXED IMMUNODEFICIENCY, ATYPICAL MYCOBACTERIA AND MYELOFIBROSIS

P. Matthioli Luis,[1] H. Ramos,[2] M.O. Freitas,[3] T. Almeida,[3] L. Rosado[3]

[1]*Hospital de Santa Maria, LISBOA, Portugal;* [2]*Hospital do Esprito Santo, VORA, Portugal;* [3]*Hospital Dona Estefnia, LISBOA, Portugal*

Background and Aims. Myelofibrosis can be idiopathic (a chronic myeloproliferative syndrome) or secondary to many kinds of insults, as a reaction to malignancy, infections, endocrinopathies, auto-immune diseases and granulomatous disease. Pancytopenia and hepato-splenomegaly are frequent, and only reversible when the secondary injury can be treated. The authors present a case of myelofibrosis diagnosed in an 11 months old boy; later it was discovered to be secondary to atypical mycobacteria and quadruple antibacillar therapy for one year reverted the clinical status. *Methods/Clinical Case*: A caucasian male infant 4 months old presents with anaemia and pneumonia with pleural effusion. Three months later, he has a peri-anal abscess and presents with hepato-splenomegaly and pancytopenia. The analysis revealed Hb 8,0g/dL (with presence of erythroblasts, frank anisopoikylocytosis, and dacriocytes), leukocytes 1500/mm^3 (absolute neutropenia 150/mm^3, left shift and basophilia), platelets 65000/mm^3; LDH 632 U/L. Bone marrow smear and biopsy showed severe myelofibrosis (without cytogenetic alterations). The thorough studies also demonstrated a mixed immunodeficiency (hypogammalobulinemia, lymphocytopenia, inversion in the relation $CD4^+/CD8^+$) and an auto-immune phenomenon (presence of auto antibodies anti-platelets and anti-granulocytes). The boy was given G-CSF on alternate days (10 μg/kg), immunoglobulin on a mensal basis and co-trimoxazole as prophylaxis for infections. With two years and 6 months old, he had already pericarditis, otitis with tympanic perforation, cutaneous mycosis, several gastro-enteritis and two pyelonephritis. Although he presented palpable lymph nodes in several areas, a liver palpable 3 cm beyond costal grid and a spleen palpable 6 cm beyond costal grid, his physical and somatometric development were normal for his age. The clinical status degrades with a growing spleen (surpassing the iliac crest) and worsening pancytopenia (Hb 6,9g/dL; leukocytes 1000/mm^3; platelets 50.000/mm^3), with muco-cutaneous blood discrasia and signs of extra-medullar hematopoiesis on both kidneys. As he has no brothers, a search was begun in the international panel for bone marrow transplantation. It was stopped by the decision for splenectomy (4 years old). The spleen showed numerous tuberculoid granulomes without caseous necrosis and although the research for typical mycobacteria was negative, he began quadruple therapy (isoniazide, rifampicine, pirazinamide and ethambutol). After splenectomy, there was normalization of haemoglobin and of platelet number; the leukocytes didn't rise as much (2000-3000/mm3), maintaining absolute neutropenia. A bone marrow smear revealed recuperation of the three haematopoietic series. Immunoglobulin therapy was suspended 2 years after splenectomy; with co-trimoxazol and penicillin prophylaxis, at 11 years old, he doesn't have a significative number of bacterial infections. Analytically, he presents Hb 13,7g/dL, leukocytes 2000/mm^3 (neutrophils 120/mm^3), platelets 662.000/mm^3. *Conclusions.* Although one of the causes indicated for secondary myelofibrosis is granulomatous disease, there aren't any published cases reporting myelofibrosis secondary to typical or atypical mycobacteria, whether in immunocompetent or immunodepressed individuals. Also it is not usual to see extra-medullar haematopoiesis on both kidneys, causing enlargement and loss of differentiation cortico-medullar. Finally, both splenectomy and anti-bacillar therapeutic were decisive in the regression of the clinical state, being the remaining neutropenia a manifestation of the immunodeficiency syndrome.

1471

PLATELET FUNCTION EXAMINATION IN ESSENTIAL THROMBOCYTHEMIA

M.P. Penka, J.K. Kissova, A.B. Bulikova, J.Z. Zavrelova, L.K. Kovarova, M.M. Matyskova

Masaryk University Hospital, BRNO, Czech Republic

Backgrounds. Basic diagnosis of essential thrombocythemia is proved by estimation of elevated platelets count and corresponding findings of activated megakaryopoiesis in bone marrow with contemporary excluding their reactive changes. The therapy is mainly focused on correction of high platelets count in the blood. The treatment is different in young and elderly patiens, in cardiovascular or thrombotic risk and non-risk patiens. However, the treatment influences not only the count, but also the function of platelets and so can lead to influencing of clinical symptoms. The function of platelets is not currently investigated before drug adminstration and in the course of the treatment. Aim of the study was to evaluate clinical and laboratory importance of platelet functional characteristics in essential thrombocythemia. *Methods.* 30 patients have been included in our observation and we performed (beside the basic laboratory tests) platelets aggregation according to Born, PFA-100 examination and flow-cytometric estimation of CD36, CD42a, CD61, CD62, CD63 markers. The laboratory testing was done before and six months after the treatment. (The platelet aggregation was tested using ADP in two concentrations, colagen and cationic propylgalat as inductors, and three parameters - percentil of aggregation, slope and desaggregation remark - were evalutated). *Results.* the decreasing of aggregation response (in 16 cases after all inductors used) was not accompaned by statisticaly significant changes in other examinations of platelet function tests. Moreover, there were not observed statisticaly significant changes in repeated examinations after six month of the treatment. There was even no correlation between functional examination of the platelets and clinical symptoms of the disease. *Conclusion*: Functional disorder of the platelets seems to be the part of clinical findings of the disease, but does not correspond with biological activity of the disease or with its clinical symptoms and/or with the answer to the therapy. Although, the treatment (especially with acid acetylosalicylic-ASA) can widely modify platelet function, it was not even observed to be significantly different in our ASA-treated vs. ASA-nontreated patients.

1472

POLYCYTEMIA VERA INITIALLY DIGNOSED AS ESSENTIAL THROMBOCYTEMIA

M. Badea, D. Badea

University of Medicine and Pharmacy, CRAIOVA, Romania

Backgrounds. The differentiated diagnosis as part of the myeloid proliferate chronic disease Ph- remains afterwards difficult, in spite of the assays to identify a sensitive and reproductive set of clinic and biologic parameters (ex. WHO). *Aims.* The aims of this piece of work are to present our experience concerning the difference between ET and PV at diagnosis. *Method*: The study contains a number of 38 patients with PV diagnosed in our clinic during the period of 1988-2005. 3 of those cases whose age was 46, 54, and 60 years old were diagnosed initially with ET. *Results.* The value of Hb was 15, respectively 16,5g/dl with Ht 44 respectively 46% for the 2 female cases and 17g/dl respectively 51% for male patients. 2 patients presented at diagnosis, leukocytes under 10.000/mm, only a case from the three of them presented more than 12.000/ mm^3. The spleen varied between 1, 5 and 2, 5 cm under the costal board. At the beginning, the value of Hb and Ht did not allow the diagnosis of PV and the high count of the platelets (650 0000, 74 0000 and 82 0000/mm^3) imposed the diagnosis of ET. The bone marrow examination was applied (after 2002) for only a patient, releasing on bone marrow biopsy: hypercellularity with hyperplasia of all marrow elements, with left deviation of the erythrocyte clusters, a polymorph megakaryocytic aspect, and that's why it was considered unclassified myeloproliferative disorder. The evolution of those three patients was in 8-18 months towards a classic PV with high levels of Hb and Ht who needed phlebotomy. *Conclusions.* At the point of prognostic and the therapeutically view of the integration in TE or PV has a low clinical importance taking into consideration that the similitude of the therapeutically approach for these 2 chronic diseases. Recently the difference becomes more important at point of molecular view (TH V617FC), even possible prognostic (ET after 10 years of evolution). The histological

study of the marrow and the evaluation of the new biological markers can chunk the diagnosis even at the beginning of the diseases

1473

SUCCESSFUL TREATMENT OF CHILDHOOD IDIOPATHIC MYELOFIBROSIS WITH STEROID

C. Khayat Djambas, P. Abou Jaoude, J. Bachir

Hotel Dieu de France hospital, BEIRUT, Lebanon

Idiopathic myelofibrosis can develop in children as well as adults. However, the disease seems to be different from adults requiring a more conservative approach to management. It is less commonly seen than adults and appears to be less aggressive, being characterized by a variable outcome reported in literature from aggressive course and a high mortality to less aggressive course and even spontaneous regression. We reported the case of idiopathic myelofibrosis in the early childhood of a boy successfully treated with standard doses of steroid. A 4 month-old boy was admitted because of severe anemia with reticulocytopenia, aniso-poikilocytosis, leukoerythroblasts, teardrop-shaped red cells and splenomegaly. The marrow was very difficult to aspirate. The bone marrow biopsy revealed reticulinic myelofibrosis. Cytogenetic study of the marrow was negative. The condition worsened with development of severe thrombocytopenia. Investigations done repeatedly ruled out malignant hemopathy, metastatic infiltration of the marrow, myelodysplasia, osteopathy, lupus erythematosis, immune disease and Fanconi anemia. After 5 months and 3 red cells transfusion prednisone therapy was attempted at 2 mg/kg/d. A complete improvement of hematological and clinical findings was observed after a month and a half. He is now 13 month old on 1m/kg/d of steroid with a hemoglobin of 14g% and platelets around 410000/mm^3.

1474

QT DISPERSION AFTER ADMINISTRATION OF RAPID INTRAVENOUS VARIOUS ANTIEMETICS IN CHILDREN PRIOR TO CHEMOTHERAPY

C. Ucar,[1] B. Oran,[2] U. Çaliskan,[2] S. Karaaslan[2]

[1]*Selcuk University Meram Medical Faculty, KONYA, Turkey;*
[2]*Seluk University, KONYA, Turkey*

Backgrounds. Children with acute leukemia are at an increased risk of cardiac arrhythmias, from their cardiac infiltrations and cardiotoxic treatments. There are many reasons why the children with acute leukemia is at increased risk of potentially life-threatening cardiac arrhythmias. The autonomic response to chemotherapy and radiation therapy (nausea, retching and vomiting) or their biochemical ejects (vomiting-induced electrolyte disturbance) can have important implications. It is, therefore, important to ensure that any medications to-administered to the children, do not further increase the risk of cardiac complications, particularly arrhythmias. Nausea and vomiting are considered to be the most distressing and debilitating side effects of therapy, and can profoundly affect patients' quality of life. *Aim and Methods.* The aim of this study was to determine the effects of the rapid administration of intravenous tropisetron, granisetron and ondansetron on measures of cardiac depolarization in children receiving chemotherapy for acute leukemia, by comparing twelve-lead ECGs before (baseline) and after 2nd and 24th hours after the drug administration. *Results.* The study was performed in total 75 children with acute leukemia (25 children for each antiemetic). QT dispersion was calculated as the difference between the maximum and minimum QTc in twelve-lead surface electrocardiogram lead. *Summary/Conclusions.* It was concluded that no clinically important cardiovascular side effects are associated with the administration of tropisetron, granisetron and ondansetron following first 24 hour. There are no dysrhythmic or hemodynamic changes in all patient groups.

1475

EFFECT OF RECOMBINANT ACTIVATED FACTOR VII ON VOLUME OF BLOOD TRANSFUSION AT PATIENTS UNDER CPB WITH NOT SURGICAL BLEEDING

M. Charnaya

Russian Research Center of Surgery, MOSCOW, Russian Federation

Backgrounds. to estimate effect rfVIIa on reduction of blood transfusion at patients under CPB with not surgical bleeding. *Materials and methods.* We surveyed 60 pts under CPB with diffuse bleeding 9,0±3,3 ml/mines (from 3,0 up to 20,0 mL / mines): group 1 (n=34) - single bolus administration rfVIIa i.v. 75,3±10,1 mkg/kg of weight; group 2 (n=26) - group of comparison. Estimated volumes of autoblood (mL), donor erythrocytes (mL), washed red autoblood cells (mL), fresh-frozen plasma (mL) separately before use of a preparation. *Results.* In group 1 through 30 mines after administration rfVIIa bleeding has essentially decreased, and in 2 hours it has completely stopped. In group 2 bleeding was kept till 12 hours after operation. Up to administration rfVIIa autoblood it was poured 11% of pts, and after administration - 0%, red blood cells - 67 and 39%, washed erythrocytes - 33 and 0%, FFP - 78 and 50%, respectively. In 38% of cases was not required any blood transfusions. Authentic distinctions in quantities autoblood (795,0±21,7 and 906,7±45,8 mL, p<0,05), red autoblood cells (354,3±94,5 and 881,2±82,6 mL, p<0,05) and washed erythrocytes (295,6±122,2 and 611,4±93,7 ml, p<0,001) between groups 1 and 2 are revealed. *Conclusion*: Use rfVIIa in therapy of uncontrollable not surgical bleedings at CPB results in significant reduction of frequency of use and volumes blood transfusion.

1476

ANALYSIS OF THE EFFECTIVE AGENTS IN HEPATITIS C VIRUS INFECTION AMONG HEMOPHILIC PATIENTS TREATED IN HEMOPHILIA CENTER OF ISFAHAN-IRAN

F. Derakhshan, M. Mojtabavi Naini, H. Hoorfar, F. Makarian Rajabi, R. Derakhshan

Isfahan University of Medical Sciences, ISFAHAN, Iran

Backgrounds. Patients with hemophilia are at high risk of post-transfusion hepatitis because of widespread use of plasma-derived products. As a consequence, hepatitis C virus (HCV) is the most common cause of chronic liver disease among hemophilic patients. *Aim*: The objectives of this study are to determine HCV prevalence, and analyze the effective agents in HCV infection in hemophilic patients. *Method*: all patients with inherited coagulation disorders registered in hemophilia center of Isfahan (553 persons) were checked for HBsAg and anti-HCV, using a third-generation enzyme-linked immunosorbent assay (ELISA) test. Positive tests for anti-HCV were confirmed by RT-PCR. Clinical history, Laboratory and treatment data of all cases were studied in January 2006. *Results.* From 465 men and 88 women with inherited coagulation disorders with Mean±SD age of 23.4 ±12.9 years,125 patients (22.6%) were HCV positive, 2 (0.4%) were HBV positive and one(0.2%) was both HCV and HBV positive. In this study the chance of coloration (with percentage correct of 72.4%) between HCV infection and cryoprecipitate usage was 5.31, between HCV and FFP usage was 3.18 and between HCV infection and moderate and severe hemophilia were 3.9 and 2.65 respectively. In this study blood group, factor concentrate consumption, age and sex of patients have no predictive value in HCV infection. 44.4% of patients with factor inhibitor were HCV positive. (p=0.006). *Conclusion*: Considering the high chance of HCV infection after transfusion of Cryoprecipitate and FFP, a more careful pre-transfusion screening of blood for anti-HCV must be introduced in all blood banks. The usage of FFP which has less chance of HCV infection, instead of cryoprecipitate in patients who do not have volume restrictions may be preferable.

1477

CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF THROMBOCYTOPATHIES IN CHILDREN IN KAZAKHSTAN

G.T. Tashenova, B.Z.H. Aldaniarova, K.O. Omarova

Scientific Center of Pediatrics and Chil, ALMATY, Kazakhstan

The problem of bleedings in childhood is one of the most actual. Thrombocytopathies (TP) - the group of diseases characterized by platelet dysfunction with predominantly microvascular type of bleeding. In structure of hemorrhagic diatheses TP has leading position (80%). The aim of this study was to assess the clinical and laboratory peculiarities of TP in 105 children hospitalized in hematological department of the Scientific center of pediatrics and children's surgery. Diagnostic complex was included anamnestic data, duration and character of the bleeding, platelet number and coagulogramm. Genealogical anamnesis on bleeding was aggravated in 75% of patients with predominantly autosomal-dominant inheritance. In 15 patients parents were examined. Hereditary TP was diagnosed in 95 (91%) patients, acquired TP in 10 (9%), out of them: drug-induced in 4 (3,5%), post-infectious in 4 (3,5%), due to endocrynopathia (hypoestrogenia) in 2 (1%). Hemorrhagic syndrome was primarily diagnosed in earlier childhood in 57 patients (54%), frequency of the relapses was increased to 10-12 y.o. in 77 patients (73%), to 15-16 y.o. bleeding symptoms regressed. Patients were divided into groups: I group - with deficit of plasma adhesion and aggregation factors (von Willebrand disease, vWD and afibrinigenemia, aF) and II group - with platelet factors alterations (inherited TP). Patients of the I group had complaints: nasal bleedings (80%) and skin hemorrhages (34%). Patients of the II group had less complaints: nasal bleedings (58%)

or skin hemorrhages (24,3%), in 43% children were directed by other specialists due to petechia appearing after procedures, anemia or others. Perinatal pathology (skin hemorrhagic syndrome, umbilical hemorrhages, intranatal intraventricular hemorrhages) was more frequent in I group (54,5%) in comparison with II group (27,3%). Bleeding time was increased in 98,8% of patients. Alterations in adhesive and aggregative platelet functions were noted in 37,4% (ristocetin-induced platelet aggregation test). Moderate platelet number decreasing, large platelet sizes (up to 7-8 μm) were noted in 8,1% of patients. In 30 patients changes in coagulogramm were observed: alteration of APTT, vWF activity decreasing (lower than 75%) and plasma vWF level (lower than 80%). In 62,6% alterations in platelet aggregation were observed: in 32 patients - an absence of collagen-induced platelet aggregation, in the rest - adenosine-diphosphate- and adrenaline-induced platelet aggregation decreasing. Prominent alteration of clot retraction was revealed in 11 patients. Thus, dysfunctions in primary chain of hemostasis in our patients were predominantly inherited, and clinical manifestation was comparatively more severe. In structure of hemostasiopathies thrombocytopathies of releasing (52,2%) and von Willebrand disease (29,3%) were prevailed, which, possibly, can be explained by dominant inheritance. Any signs of bleeding diatheses in children are should be approached and require specialized investigations, including examination of relatives with aggravated anamnesis on bleeding.

1478

NASAL BLEEDING IN CHILDREN WITH HEREDITARY THROMBOCYTOPATHIAS

B.Z.H. Aldaniarova, K.A. Mazhibayev, G.T. Tashenova, K.O. Omarova

Scientific Center of Pediatrics and Children, ALMATY, Kazakhstan

Nasal bleeding in children is not rare pathologic condition, which causes diagnostic and therapeutic difficulties among physicians. Bleeding from nasal cavity is not a disease, but the symptom of the local or systemic disease. The most intensive and severe nasal bleeding more often has place in cases of hemorrhagic diathesis. Hemorrhagic diatheses are characterized by hereditary, congenital or acquired system bleeding disorders. According on data of various investigators, from 40% to 80% of all cases of bleeding disorders related with quantitative and qualitative disorders of thrombocytic hemostasis. Among thrombocyte-dependent hemostasis disorders the special interest is directed to thrombocytopathias due to fact that 80% of hemorrhagic diatheses are related with disorders of primary (thrombocytic) stage of hemostasis. However, the clinical manifestations of the majority of hemostasiopathies are monotypic, which makes the diagnostic difficulties. Earlier establishment of the cause of hemostasis disorder is necessary for administration of an adequate hemostatic therapy. Under our observation were 42 children aged from 1 to 15 years old with thrombocytipathies, hospitalized to oncohematologic center in SCPCS. The diagnosis was based on anamnesis, clinical manifestation, laboratory data. Assessment of hemostasis system was based on the results of standard investigations: platelet number, time of bleeding, coagulation time, prothrombin time, activated partial thromboplastin time, thrombin time, total blood fibrinogen level, functional activity of platelets: adghesive and aggregative functions in vitro and aggregometry. Investigation of factor von Willebrand activity was conducted in 'HEM' company. Moreover, all children were consulted by neurologist and otolarhyngologist. In result the of analysis the following types of hemostasiopathias were revealed: releasing thrombocytipathias in 27 (64%) patients, von Willebrand disease in 11 (26%) and Glanzman's thrombastenia in 4 (10%). Out of them in 31 (74%) patients relapsing nasal bleeding was noted. The first bleeding manifestation in 17 (40,5%) was noted from the birth with the high frequency of bleeding up to 3 years old. The majority - 24 (57%) of patients were boys. Hereditary character of the disease was in 76%, acquired forms was established in 24% of patients. In majority of cases nasal bleeding was combined with other localizations (petechias, echimoses, gingival bleeding, metrorrhages, post-traumatic and post-injection bleeding, bleeding from ears and hemorrhages into sclera). Hematomas and hemartroses typical for hematomic type of bleeding disorders were noted in 2 patients with von Willebrand disease. In result of laboratory investigations normal number of platelets was noted and this indicator doesn't change during the study period. An assessment of Duke's bleeding time showed prolongation in 83% of patients (more than 4 min). The time of coagulation in all patients was normal 96-10 min). Prolongation of bleeding time shows defect in platelet, but not coagulative hemostasis. To reveal platelet dysfunction we have assessed an adhesive function of platelets. An adhesion of platelets to glass was assessed in 21 children, out of them in 18 (85,7%) adhesive dysfunction was revealed and was 0-20% in comparison with normal 30-40%. To exclude coagulative disorders we have assessed standard indicators of hemostasis system: coagulation time, prothrombin time, activated partial thrombplastin time, thrombin time, blood fibrinogen level, von Willebrand factor. Generally, all those indicators was in normal ranges. An assessment of aggregative function of platelets showed decreased aggregation to ristomycin in 19% patients and an absence of aggregation in 7%. An absence of adenosine-diphosphate-induced aggregation in dose 2,5 mg/ml, adrenalin-induced aggregation in dose 1 mg/ml was established in 19% of patients. After assessment of clot retraction, the decreasing lower than 40% was noted in 4 patients, and Glanzman's thrombastenia diagnosis was established. The decreasing of the secondary aggregation (second wave) induced with adenosine-diphosphate and adrenalin was established in 38% of patients. The diagnosis of von Willebrand disease was established in 26% of patients with normal platelet number, increased activated partial thromboplastin time (over 55 sec), decreased activity of von Willebrand factor (lower than 80%). Neurologist has established vegetative vascular dystonia and intracranial hypertension in 12 patients. In 11 patients otolarhyngologist has revealed during the rhynoscopy the superficial localization of blood vessels, especially in Kisselbakh zone. Thus, nasal bleedings in children with thrombocytopathias - the most often symptom. Bleeding in form of petechias, echimoses, gingival, nasal bleeding in children with normal or slightly decreased platelet number may be due to qualitative disorders of platelets. Bleeding in form of hematomas, hemarthroses reveals double defect in hemostasis, typical for von Willebrand disease. After establishment of bleeding type laboratory investigations to reveal character of hemostasiopathy are necessary. Vegetative vascular dystonia, intracranial hypertension, vascular changes in nasal mucosa, possibly, may play role in relapses of nasal hemorrhages in patients with hemostasis disorders. Earlier establishment of the cause of nasal bleedings will allow to conduct an adequate therapy.

1479

DERANGEMENT OF HAEMOSTATIC PROTEINS IN HCV CIRRHOTIC PATIENTS: RELEVANCE TO HEMORRHAGIC DIATHESIS AND THROMBOTIC EPISODES

E.I. El-Bassiouni, A.E. El Bassiouny, A.A. Taha, H.R. El Khayat

Theodor Bilharz Research Institute, GIZA, Egypt

Backgrounds. An altered coagulation profile resulting in decreased natural anticoagulant levels leading to haemostatic activation is described in patients with liver cirrhosis. The protein C system, a major physiologic regulator of haemostatic balance, controls thrombin production and guards against thrombotic episodes. Aims. This study was designed to assess the components of protein C system in HCV cirrhotic patients to determine whether alterations in these haemostatic proteins are related to degree of hepatic dysfunction and/or haemostatic activation and development of hemorrhagic diathesis or thrombotic episodes. Methods. Components of protein C (PC) system were assessed in 44 patients with hepatitis C virus liver cirrhosis, of whom 15 patients had acute hematemesis and 14 patients had portal vein thrombosis (PVT). According to Child-Pugh criteria, all patients were graded Child C. Neutrophil elastase (NE) release was determined by measuring elastase-α-1-proteinase inhibitor (E-α-1-PI) complex using an immune activation assay. Levels of tumor necrosis factor-α (TNF-α), PC antigen (PC Ag), total protein S (TPS), free protein S (FPS), soluble thrombomodulin (TM), tissue-plasminogen activator (t-PA), t-PA-PAI-1, plasmin-α-2-antiplasmin (PAP), thrombin-antithrombin III (TAT) and D-dimer (D-D) complexes were measured in plasma by ELISA. Fibrinogen level, functional activities of PC (PC Ft), plasminogen activator inhibitor-1 (PAI-1) and C4b-binding protein (C4b-BP) concentrations were also assessed. Results. Stimulation of the inflammatory process (increased TNF-α, NE and C4b-BP), endothelial injury (elevated TM and t-PA), reduction in anticoagulant proteins (low PC and PS), hypercoagulation and thrombin generation (elevated TAT and D-D), increased consumption (prolongation of coagulation screening tests, thrombocytopenia, hypofibrinogenaemia and decreased PC Ft/PC Ag ratio) and accelerated fibrinolysis (increased PAP, free t-PA and t-PA/PAI-1 ratio and decreased PAI-1) were detected in different cirrhotic groups compared to controls (15 healthy subjects). The haemostatic defects correlated with the marked elevation of inflammatory mediators and were more pronounced ($p<0.05$) in patients with PVT. A significant decline ($p<0.05$) in fibrinogen concentration and PC Ft/PC Ag ratio associated with a significant increase ($p<0.05$) in TAT and D-D levels was detected in bleeders with acute hematemesis and patients with PVT compared to cirrhotics with haemostatic balance. Moreover, FPS and PAI-1 levels were significantly elevated ($p<0.05$) in patients with PVT compared to those with acute hematemesis and were

inversely correlated with platelet count (p<0.01). *Conclusions.* These findings suggest that NE and TNF-α contribute to haemostatic alterations in patients with viral hepatitis C liver cirrhosis, and emphasize the clinical significance of protein C as a sensitive parameter for hepatic dysfunction and protein S and PAI-1 as reliable prethrombotic markers in these patients.

1480

FX DEFICIENCY IN A GREEK FAMILY

P.P. Paraskevopoulou, A.Vlahopoulou, A.Mpenekou, M.Katelani

Hematology Laboratory G, Hospital of Athens 'Agia Olga'; Greece

FX deficiency is an hereditary disease and is one of the rarest factors deficiencies with incidence for homozygous type 1/1.000.000 in the general population.Only 50 cases have been reported all over the world.People can suffer from bleeding when the FX level is below 10%. The symptoms varies from mild to severe bleeding.The heterozygous type is commonly asymptomatic. A young patient 17 years old investigated in our laboratory in order to take oral contraceptives because she had prolonged bleeding during menstruation. We found slightly prolonged PT and APTT and she was further investigated for TT, Fibrinogen, DDimers, FDP's, full blood count and full biochemical tests. Her parents and her three brothers and the sister of her father and her 2 children were also investigated. *Results.* The tests of the patient PT:16.3 sec (, INR:1.3, APTT:40.2sec. Then the plasma was diluited 1/1 with normal plasma and then was measured immediately and 1 and 2 hours after incubation in 37°C waterbath. In all the measurements the tests were in normal range.The measurement of the factors FVII, FIX, FX,FXI,FV,FVW is normal except the FX: 46.4% (normal range 70 - 140%). The mother of the patient was normal of all tests. The father had PT:15.1sec, INR:1.24, APTT:38.6sec and FX: 50.5%. The two brothers were normal of all the tests.The third brother had PT:16.2sec, INR:1.33, APTT:42.9sec and FX: 36.8%. The sister of the father had PT:14.5sec, INR:1.14, APTT:37.9sec and FX: 55.6%. From her children the first was normal and the second had PT:15sec, INR:1.33, APTT:37.8sec and FX : 54.9%. The human FX gene is located on chromosome 13q34 and it is an 'autosomal recessive' disorder.The half life of the factorX is 40-45 hours. The treatments to control bleeding is FFP and Prothrombin Complex Concentrate

1481

THE INFLUENCE OF STORAGE AND LEUKOCYTE DEPLETION ON THE ANTIGEN DENSITY IN DUFFY AND MNS BLOOD SYSTEMS MEASURED BY FLOW CYTOMETRY

G.H. Lorand-Metze, S.B. Calonego, M.L. Barjas-Castro, F.G. Pereira, K. Metze

State University of Campinas, CAMPINAS, Brazil

Backgrounds. Red blood cell (RBCs) antigens are polymorfic structures located in the RBC membrane. Fya and Fyb antigens are the most important ones in the Duffy system and they are carried by multipass transmembrane glycoproteins. S and s antigens, belonging to MNS system, are carried by glycophorin B (GPB). These RBC antigens are sensitive to enzymes produced by leukocytes, and therefore can present changes in expression during the storage period. The behavior of erythrocyte antigen expressions during storage period is an important marker in the quality control of RBC reagents used in Transfusion Centers. These data can also help to define the RBC reagents validity time. *Aims.* to evaluate the influence of leukocyte enzymes and storage in the expression of Fya, Fyb, S and s antigens of RBC units collected with CPDA-1. *Methods.* We studied 49 RBC units, which had been divided into two sub-units immediately after blood collection. One was submitted to a leukocyte reduction using SepacellTM filters before storage and the other was used as a control. Evaluation of antigens was carried out on days 1 and 35 of the storage period using hemagglutination techniques (tube and gel tests) and immunophenotyping by flow cytometry. The determination of the number of antigenic sites for each antigen studied was performed by flow cytometry (FCM) using the QIFIKITTM for standadization. *Results.* Concerning the influence of leukocyte depletion, only Fya presented an increase in the antigen fluorescence intensity (p=0,02) on day 35 of storage in the leukocyte-depleted bag. The other antigens (Fyb, S and s) presented no difference of expression after leukocyte-reduction. The storage period of 35 days did not affect the antigen density of Fya, Fyb, S and s. Concerning the number of antigenic sites, Fya showed a median number of 1,1 and 2,3 x104 in donors Fy(a+b+) and Fy(a+b-) respectively; Fyb 0,72 and 0,78 x 104 in donors Fy(a+b+) and Fy(a-b+); S 1,0 and 2,1 x 104 in individuals Ss and SS and antigen s showed 1,0 and 2,4 x 105 sites in donors Ss and ss respectively. *Conclusion*: FCM showed to be an efficient technique, able to detect antigen small expression alterations that were imperceptible with other methods. This technique was more reproducible, stable and appropriate for Fya and Fyb antigens than for S and s antigens, probably due to the characteristics of the commercially available anti-S and anti-s antibodies. The leukocyte-reduction only influenced Fya antigen on day 35 of storage.

1482

COMPARATIVE STUDY OF THREE CD34+ CELL SELECTION DEVICES IN AUTOLOGOUS STEM CELL TRANSPLANTATION SETTING: ISOLEX 1.2 (BAXTER), ISOLEX 2.5 (BAXTER) AND CLINIMACS (MILTENYI)

M.J. Rodriguez Salazar, R.F. Rodriguez Snchez, B.J. González González, M.T. Hernández Garca, J.M. Raya, G. González Brito, J.A. Rodriguez López, F. Hernández Mndez, L. Hernández Nieto

Hospital Universitario de Canarias, La Laguna - SANTA CRUZ DE TENERIFE, Spain

In the setting of autologous stem cell transplantation, several studies suggest tumour-free grafts may improve outcome. Positive CD34+ selection of PBSC (peripheral blood stem cell) can be used as a purging strategy, reducing tumor cell contamination of the graft. To compare purging efficiency and recovery data obtained with three distinct devices. We compared the results of 46 CD34+ cell positive selection processes performed in our center with three distinct devices: Isolex 1.2 (9 cases), Isolex 2.5 (18 cases) and CliniMACS (19 cases). There was no statistical difference between the three groups neither in terms of number of total nucleates cells, number of total CD34+ cells nor in percentage of CD34+ cells. The characteristics of the CD34+ cell selection column (purity and recovery) obtained with each system were the following: 1) Purity (%): Isolex 1.2: 90,21 (67,5 - 99,3); Isolex 2.5: 97,98 (99,4 - 94,6) and CliniMACS: 97,10 (95,0 - 98.8); 2) Recovery (%): Isolex 1.2: 42,58 (32,93 - 67,80) Isolex 2.5: 65,59 (43,78 - 89,33) and CliniMACS 59,96 (39,96 - 79.96). We compared purity and recovery obtained by the three immunoselección devices (Table 1). In our experience, with the system Isolex 1.2 we obtained a statistically significant poorer recovery and purity of the CD34+ enriched cells as compared with the other two devices. We did not find significant differences between version 2.5 of the Isolex and the CliniMACS, neither in the purity of the final product, nor in the recovery of cells CD34+.

Table 1.

Purity	*Recovery*
Isolex 1.2 vs Isolex 2.5 (p:0,009)	Isolex 1.2 vs Isolex 2.5 (p:0,001)
Isolex 1.2 vs CliniMACS (p:0,031)	Isolex 1.2 vs CliniMACS (p:0,05)
Isolex 2.5 vs CliniMACS (p:0,141)	Isolex 2.5 vs CliniMACS (p:0,127)

1483

ERYTHROEXCHANGE BY FRESENIUSCOM TEC TO TREAT ACUTE PAIN CRISIS IN SICKLE-CELL DISEASE. A CASE REPORT

P. Bergamaschi,[1] C. Perotti,[2] C. Del Fante,[2] G.L. Viarengo,[2] T. Santini,[2] G. Ippoliti,[3] A. Marchesi,[2] C. Parisi,[2] L. Salvaneschi[2]

[1]*IRCCS Policlinico 'San Matteo', PAVIA, Italy;* [2]*IRCCS Policlinico 'San Matteo', PAVIA, Italy;* [3]*Internal Medicine, Ospedale Civile, VOGHERA, Italy*

Background and Aims. homozygote patients for sickle-cell disease (SCD) have abnormal hemoglobin which undergoes gradual reversible polymerization and aggregation. Repetition of this process may lead to irreversible membrane changes and characteristic sickle cell morphology. Red blood cells (RBC) containing haemoglobin S (HbS) are less deformable thereby affecting blood viscosity and predisposing to episodes of tissue infarction by microvascular thrombi. Occlusions in microcirculation clinically manifest as crisis, the most common being classic pain crisis, involving severe musculoskeletal, thoracic and abdominal discomfort. In addition to pain control, hydroxyurea to increase HbF and supportive care, RBC transfusion is the standard approach with the aim of improving oxygen transport to tissues and diluting sickle cells. Therapeutic erythroexchange (TREX) replaces sickle cells with large amount of normal erythrocytes. Despite TREX has never been shown to influence significatively the course of pain crisis, it seems a reasonable approach to treat severe acute SCD complications. Furthermore, TREX presents several advantages over direct transfusion increasing HbA

quickly with concomitant HbS removal and ameliorating hyperviscosity. We report about SCD acute painful crisis in a 25-years-old woman, who benefit by automated TREX performed at our Apheresis Service using Fresenius™ COM.TEC device with a new dedicated program.

Table 1. Characteristics of the patient.

	1st TREX		*2nd TREX*	
	PRE	*POST*	*PRE*	*POST*
Pain (black, abdominal, legs)	intense	reduced	mild	no
Morphine	i.v. continuously	suspended	no	no
Hct (%)/ Hb (g/dL)	26.0/9.2	27.8/9.6	27.7/9.0	30.2/10.5
Hbs (%)	80	28	45	19

Table 2. Characteristics of eritroexchange.

	1st TREX	*2nd TREX*
No./total volume of RBCs* (mL)	8/1955	7/1823
Mean Ht(%) of RBCS* (mL)	56	57
Patient blood volume (mL)	3710	3710
Processed blood volume (ML)	3997	3329
Anticoagulant to the patient (mL)	282	239
Flow rate (mL/min)	37	40
Time of procedure (min)	112	93

RBCs* = red blood cell units

Methods. a blood specimen was drawn in advance to assess compatibility, then cross-matched RBC units were filtered for leukocyte depletion. A detailed informed consent was obtained. Erythroexchange was performed by double-vein technique using a new program (Fresenius HemoCare™, Bad Homburg, Germany) which permits to predict both the final hematocrit and HbS level of the patient. Blood cell count and HbS percentages by current haemoglobin electrophoresis were measured before and after each TREX. The pre-apheresis HbS value permitted to appropriately set the cell separator program with the goal of reducing HbS to less than 30%. Check of post-apheresis HbS allowed verifying the accuracy of instrument predictions. During the procedure the patient was carefully monitored as respect to vital signs (blood pressure, heart rate, oxygen saturation) and occurrence of adverse events, in particular signs of transfusion reaction. Calcium gluconate was administered i.v. to prevent or minimize citrate toxicity. *Results.* we performed two TREX procedures on alternate days; the relevant data are given in the table 1 and 2. Complete clinical remission was obtained with no evidence of alloimmunization or other serious complications. *Conclusions.* our experience confirms the beneficial effects of TREX for SCD pain crisis especially when isovolumetric procedures are carried out with an automated device.

1484

PREHARVEST PREDICTIONS OF STEM CELL PRODUCTS

R. Gilli, S. Sipurzynski, S. Macher, K. Rosskopf, W. Helmberg, G. Lanzer

Univ. Klinikum Graz, GRAZ, Austria

Backgrounds. Collected mobilized peripheral stem cell is the commonly used resource for autologous transplantation. The amount of CD34+ cells in the peripheral blood is used as determinant for starting collection. *Aims.* Aim was to see if not only the amount of CD34+ cells /µl determines the yield of the product but also the percentage of CD34% cells should be considered when to start with collection. *Methods.* 259 aphereses of adult patients suffering from hematological (AML, CLL, NHL, MM) disorders and children with solid tumors (ewing sarcoma, neuroblastoma, rhabdomyosarcoma,) were evaluated. Aphereses were done with the Cobe Spectra™, 3-4 times the blood volume was performed. Measured were WBC, MNC and CD34+Cells in the peripheral blood, amount of $CD34^+$cells /kgBW in the aphereses products, collected $CD34^+$cells/processed liter and efficacy of the procedures (MNCs, $CD34^+$cells.) *Results.* According to the specifications of the transplanting departments ($CD34^+$cells > $2x10^6$/kgBW + back up) 190 (73,35%) of the aphereses were completed successfully. 31 out of 190 (16,31%) successfully completed aphereses were started with $CD34^+$cells<20/µL. In 19 out of 23 aphereses we were successful with $CD34^+$cells <20/µL and >0,2%. *Conclusion*: In collections started with $CD34^+$cells <20/µL the percentage of CD34+ cells is a good predictive factor for successful apheresis, even more so for adults then for children. So don`t forget to look at the percentage of $CD34^+$Cells in peripheral blood when you decide to start with stem cell apheresis.

Table 1.

CD34+	*>0.2%*	*<0.2%*
Adults		
>20/µL	97% (104/107)	76% (23/30)
<20/µL	82% (14/17)	13% (7/53)
Children		
>20/µL	95% (20/21)	92% (12/13)
<20/µL	83% (5/5)	41% (5/12)

1485

THROMBOTIC THROMBOCYTOPENIC PURPURA POST-HEMATOPOIETIC STEM CELL TRANSPLANTATION

M.M. Marinacci,[1] A. Lanti,[2] D. Del Proposto,[2] C. Cordero,[2] M. Messina,[2] M. D'Angelosanto,[2] L. Tiberi,[2] A. Adorno,[1] I. Isacchi[3]

[1]*Tor Vergata University, ROME, Italy;* [2]*Policlinico Tor Vergata, ROME, Italy;* [3]*Bambino Gesu' Pediatric Hospital, ROME, Italy*

Thrombotic thrombocytopenic purpura (TTP) is a rare complication of hematopoietic stem cell transplantation (HSCT): the literature is scant and heterogeneous, little is known about the pathogenesis, except that it appears to differ from that of classical TTP. Plasma exchange (PE) is commonly employed for the therapy, but there are no data that support its use. We present our experience in treatment of two post-HSCT TTPs with PE. From May 2004 to December 2005, 52 patients underwent HSCT, and TTP was diagnosed in 2 of them , respectively on post transplant day 47 and 102 . Both patients received HSCT from HLA-compatible related donors. TTP was defined as the simultaneous occurrence of red cell fragmentation, laboratory findings of haemolysis with negative direct and indirect antiglobulin test, high LDH level, red cell transfusion requirement and thrombocytopenia caused by consumption, in the absence of disseminated intravascular coagulation. PEs were performed using fresh frozen plasma as replacement fluid. PE was well tolerated, but the two patients had no response to the treatment. One patient died because of fungal infections. Our experience confirm the data of the recent literature. TTP is a rare and serious complication of hematopoietic stem cell transplantation and further, systematic studies are necessary for a better knowledge of its incidence, treatment and outcome.

1486

QUALITY OF LIFE OF ONCOLOGICAL AND HEMATOONCOLOGICAL PATIENTS AFTER THE HSCT: FINDING FROM CROSS-SECTIONAL AND RETROSPECTIVE STUDY.

L. Slovacek,[1] B. Slovackova,[2] L. Jebavy,[1] J. Horacek,[1] M. Blazek[3]

[1]*University of Defence, HRADEC KRALOVE, Czech Republic;* [2]*Psychiatric Clinic, HRADEC KRALOVE, Czech Republic;* [3]*Department of Hematology, HRADEC KRALOVE, Czech Republic*

Backgrounds. The cross-sectional, retrospective and descriptive study evaluates quality of life (QoL) of patients after the hematopoietic stem cell transplantation (HSCT) at the Department of Clinical Hematology of the 2nd Internal Clinic of the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic from 2001 to 2003. Aims. 1. to verify the applicability of the Czech version of an international generic European Quality of Life Questionnaire - Version EQ-5D for the evaluation of QoL in patients after the HSCT at the Department of Clinical Hematology of the Second Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec

Kralove, Czech Republic, 2. to evaluate the QoL of patients after HSCT at the Department of Clinical Hematology of the 2nd Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic and 3. to analyse influence of selected factors on QoL of patients after the HSCT at the Department of Clinical Hematology of the Second Internal Clinic in the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic. Patients and Methods. The total number of respondents after the transplantation was 95 and the return rate of questionnaires was 72.1% (71 respondents - 39 men and 32 vomen) and we could evaluate 100% of them. Their average age was 55.5 years. We used the Czech version of an international generic European Quality of Life Questionnaire - Version EQ-5D. The influence of monitored factors (type of transplantation - autologous, allogenous, age, sex, education, polymorbidity, merital status, religion and the time lapse from the HSCT) on the QoL of patients after the HSCT was determined by means of dispersion analysis. *Results.* The above-mentioned factors proved statistically significant dependence of EQ-5D score and EQ-5 VAS on age (in both cases $p<0.01$), polymorbidity (in both cases $p<0.01$) and on religion (in both cases $p<0.01$). The influence of other factors on EQ-5D score and EQ-5D VAS was not proven as statistically significant. Conclusion: EQ-5D score (dimensions of QoL) and EQ-5D VAS (a subjective health condition) significantly decrease with increasing age and with a higher number of associated diseases. They are significantly higher in patients who believe in God compared to patient without religious beliefs. Based on our study we can further state that the QoL of our patients after the HSCT at the Department of Clinical Hematology of the Second Internal Clinic of the University Hospital and Medical Faculty of Charles University in Hradec Kralove, Czech Republic, is very high, which is seen from mean EQ-5D score (72.5%) and mean EQ-5D VAS (76.5%) values.

1487

QUALITY OF LIFE OF LITHUANIAN CHILDREN SUFFERING FROM CANCER

J. Makari,[1] A. Zaborskis[2]

[1]*Kaunas University of Medicine Hospital, KAUNAS, Lithuania;* [2]*KMU Insitute for Biomedical Research, KAUNAS, Lithuania*

Cancer is the most often cause of death in children. According to data of the Lithuanian cancer registry, in the last decade 70'100 new cases of children cancer were diagnosed yearly. In Lithuania, the quality of life of children suffering from cancer until now has not yet been evaluated properly. Aims. The aim of the study is to increase the understanding of the quality of life of Lithuanian children suffering from cancer. Methods. The study started in February of 2005 in the Division of Oncology and Hematology at the Clinical Hospital of Kaunas University of Medicine and in the Division of Oncology and Hematology at the Vilnius University Children's Hospital. During one year, 63 children aged 2'18-year and their families were invited to participate in the study. In the sample, 55% of children suffered from hematoblastosis, 13% from CNS tumors, and 32% from solid tumors of other localizations. The children and their parents were questioned within 2 to 6 weeks from the date of diagnosis. We used the PedsQL (Pediatric Quality of Life Inventory TM) questionnaire initially developed to evaluate the quality of life of children between the ages of 2 and 18. The questionnaire was translated from the original English version (designed by Dr. James Varni and the Mapi Research Institute) into Lithuanian according to the linguistic validation criteria. Children aged 5'7 were interviewed by researchers while older children and parents of children from all age groups filled out the questionnaires by themselves. *Results.* 36.1% of children aged 8'18 stated that they had low energy often or almost always; 54.9% of their parents thought similarly. Children aged 5'7 less often complained of having low energy when compared with older children; none of the children in this age group complained of always being tired. 40.0% of parents whose children were 2'4-year-old felt that children often or almost always needed more energy to play. Among 8'18-year-olds, 27.8% of respondents stated they never felt scared and sad; 22.2% of the respondents did not feel angry because of their present disease. In this age group, 9.7% and 16.1% of parents felt scared, sad and angry respectively. 33.3% of respondents stated that they sometimes felt uneasy that their disease will relapse; parents worried about this more often - 41.9%. Furthermore, in the 8'18-year age group, 27.8% of children stated feeling pain often or almost always, whereas this complaint was stated by 38.8% of their parents. Among 5'7-year olds, often or almost always felt pain was reported by 12.0% of children, whereas the child's pain was indicated by 28.9% of parents. Among 2'4-year-old children, 46.7% of parents stated that their children often felt or almost always felt pain. Conclusions. Children evaluated their quality of life as being better when compared with their parents. Younger children evaluated their quality of life as better than older children.

1488

THROMBOPHILIC MUTATIONS IN THALASSEMIA AND β(0)-THALASSEMIA

F. Kouskou,[1] L. Benetatos,[2] V. Alymara,[2] A. Bassou,[2] K.L. Bourantas[2]

[1]*University Hospital of Ioannina, IOANNINA, Greece;* [2]*Dept. of Haematology, IOANNINA, Greece*

Backgrounds. The thalassemic patients present a higher than normal incidence of thromboembolic events including cerebral thrombosis, deep venous thrombosis and pulmonary embolism. The study of the hemostasis of thalassemic patients revealed increased circulating platelet aggregates, a significant shortening of platelet life span, increased concentrations of urinary metabolites of thromboxane A2 and prostacyclin, low levels of protein C and protein S. On the other hand, a number of mutations are associated with an increased risk of thrombosis. Heterozygotes for the Factor V G1691A (Leiden) mutation experience 2-5 times the normal risk of thrombosis , while homozygotes' risk is 80 times the risk in non carriers. The MTHFR G677T and A1298C mutations are associated with homocysteinemia and increased risk of cerebrovascular disease and peripheral artery disease. Other thrombophilic mutations are the Prothrombin G20210A, FV H1299R (R2), Factor XIII V34L, β-Fibrinogen '455 G-A, PAI-1 4G-5G, GPIIIa L33P (HPA-1), ACE I/D, Apo B R3500Q and Apo E2/E3/E4. *Aims.* The aim of the present study was to check whether the presence of a thrombophilic mutation in a thalassemic patient increases the risk for the development of a thromboembolic event.

Table 1. Number and percentage of thrombophilic mutations.

	Thalassemic (20)			*Non Thalassemic (20)*		
Mutation	*Total*	*Heterozygous*	*Homozygous*	*Total*	*Heterozygous*	*Homozygous*
Factor V G1691A (Leiden)	20			20	3 (15%)	
Factor V H1299 (R2)	20	3 (15%)		20		
Prothrombin G20210A	20	3 (15%)		20	4 (20%)	
Factor XIII V34L	20	11 (55%)		20	5 (25%)	1 (5%)
B-Fibrinogen 455 G-A	20	11 (55%)	1 (5%)	20	8 (40%)	
PAI-1 4G/5G	20	15 (75%)	4 (20%)	20	10 (50%)	6 (30%)
GPIIIa L33P (HPA-1)	20	1 (5%)		20	5 (25%)	
MTHFR C677T	20	8 (40%)		20	9 (45%)	1 (5%)
MTHFR A1298C	20	7 (35%)	4 (20%)	20	13 (65%)	1 (5%)

Methods. We have screened an unselected group of 20 patients, 17 men and 3 women. 16 of them had β-thalassemia major, 2 intermediate β-thalassemia and 2 S-β(0)-thalassemia. The mean age of the patients was 30,5 years. One of the patients presented ulcer of the lateral malleolus, another had avascular necrosis of the femoral head. A group of 20 sex- and age-matched healthy individuals served as control group. DNA analysis was performed by polymerase chain reaction and reverse hybridization. Both patients and healthy individuals were checked for 9 mutations: FV G1691A (Leiden), FV H1299R (R2), Prothrombin G20210A, Factor XIII V34L, β-Fibrinogen '455 G-A, PAI-1 4G/5G, GPIIa L33P (HPA-1), MTHFR C677T, MTHFR A1298C. *Results.* Table 1 shows the number and the percentage of heterozygotes and homozygotes of the mutations that were studied. The profiles of the mutations of the two thalassemic patients that were having clinical manifestations of hypercoagulability were the following: the 34-year old man with

intermediate β-thalassemia and ulcer of the lateral malleolus was heterozygous for FXIII V34L, β-fibrinogen '455 G-A, PAI-1 4G/5G, MTHFR C677T. The 57-year old man with Sβ(0)-thalassemia and avascular necrosis of the femoral head was heterozygous for prothrombin G20210A, FXIII V34L, β-Fibrinogen '455 G-A, PAI-1 4G/5G, MTHFR A1298C. *Conclusions.* The prevalence of the thrombophilic mutations in thalassemic patients doesn't differ much of the prevalence of these mutations in non thalassemic people. However, the presentation of any of the thrombophilic mutations in a thallasemic patient is a factor that contributes, among others, to the development of a thromboembolic event.

1489

AUDIT OF INDICATIONS FOR OUT OF HOURS COAGULATION SCREENING AT THE ULSTER HOSPITAL

D.C. Wylie, M. El Agnaf

Ulster Community and Hospitals Trust, BELFAST, United Kingdom

The number of coagulation screens performed out of hours has rapidly been increasing, with a corresponding rise in the cost to laboratories, both in terms of materials and staff time. In January 2005, almost 700 coagulation screens were performed out of hours. It was felt that many of these were not clinically indicated, and most were normal. Of the abnormal results, only a small percentage were actually treated. It was therefore felt that an audit in this area was appropriate, to examine how this resource is being misused. The aims of the study were as follows: - to study the out of hours coagulation screens performed in the Ulster Hospital; to produce guidelines to be followed prior to performing the test; to rationalise the number of tests performed. 100 patients who had coagulation screens performed out of hours in January 2005 were randomly selected. The indications for the test were examined; appropriate indications included known or suspected liver disease, history of haemorrhage, current haemorrhage or renal failure. We also looked at treatment given for abnormal results. We felt that treatment should be given if the result was abnormal by 50% or more. The total number of screens performed in the one month period was 677, taken form 592 patients. Of the 100 cases we examined, 70% were in fact normal. Only 35% of the tests were clinically indicated. Only 10% of the patients who had abnormal results were treated. We concluded from the study that the majority of coagulation screens performed out of hours in the Ulster Hospital were not indicated; even if the results were abnormal, most were not acted upon. We therefore feel that local and national guidelines ought to be developed, to reduse wastage of this resource.

1490

PRE-EMPTIVE ANTIFUNGAL THERAPY IN HIGH-RISK PATIENTS WITH ACUTE LEUKEMIA: COST-EFFECTIVENESS OF INTRAVENOUS ITRACONAZOLE

M.R. Villa, S. Improta, A. Lucania, M. Sagristani, M. Esposito, G. Nitrato Izzo, F. Gonnella, L. Mastrullo

PO San Gennaro, NAPLES, Italy

Backgrounds. Systemic fungal infection remain a major clinical problem in immunocompromised patients, particulary in patients with prolonged severe neutropenia, preexisting myelodisplasia and advanced age. In these cases, presumed systemic fungal infections are treated empirically to reduce documented infections and associated mortality. *Aims.* We, retrospectively, compared the cost-effectiveness of intravenous itraconazole treatment with conventional treatment with liposomal amphotericin-B or new antifungal drug as voriconazole, in patients affected by AML or high-risk MDS. *Methods.* Since January 2003 to December 2005, 38 patients (24 female and 14 male, median age 72) affected by AML or high-risk MDS, who underwent induction chemotherapy, received primary antifungal prophylaxis. Twenty patients were treated with fluconazole 200 mg/day os and 18 patients with itraconazole 200 mg bid os. During induction therapy the median length of severe neutropenia (PMN<500/m^3) was 19 days. Febrile episodes have been emprically treated with broad-spectrum antibiotics (cephalosporine plus aminoglicoside with or without glycopeptide). Preemptive and empirical antifungal treatment for fever unresponsive to broad-spectrum antibiotic therapy was employed, after 5 days, in 20 *fluconazole* patients with liposomal amphotericin-B 3 mg/Kg/die intravenous in 9 patients or voriconazole 6 mg/Kg bid during first 24 day followed by 4 mg/kg bid intravenous in 11 patients. In the subgroup treated with oral itraconazole all patients were switched to intravenous drugs at the dose of 200 mg over 60 minutes every 12 hours during the first 2 days followed by 200 mg given i.v. once daily. All patients were treated with pre-emptive therapy for a median of 14 days (11-21). *Results.* There were no significant differences noted between the three subgroups with regard to the duration of prophylaxis (median: 10 days for fluconazole vs 11 days for oral itraconazole), percentage of patients who developed fever unresponsive to broad-spectrum antibiotic therapy (46% in fluconazole group vs 39% in itraconazole group), proven/probable or possible fungal infection as well as with regard to survival. Safety and toxicity analysis of pre-emptive treatment was similar in all subgroup, only one patients withdrawal from voriconazole therapy for hallucination and one withdrawal from intravenous itraconazole for nausea and vomiting therapy resistant. When we compared cost of three pre-emptive therapy we showed that lipid amphotericin-B and voriconazole were most expensive than intravenous itraconazole both we consider daily treatment cost and total treatment associated with nurse cost. *Conclusion*: Intravenous itraconazole has at least equivalent efficacy as empirical antifungal therapy in immunocompromised patient affected by AML or high-risk MDS. However, intravenous itraconazole compared with other antifungal treatment was shown to be the best cost-effective and cost-saving pre-emptive empirical therapy.